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FIELD EXPOSURE AND BIOLOGICAL EFFECTS OF GRAIN FARMERS DURING CARBOFURAN APPLICATION FOR GRASSHOPPER CONTROL

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EFFECTS OF ROADSIDE APPLICATION OF CARBOFURAN ON NESTING BREWER'S BLACKBIRDS

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EFFECTS OF CARBOFURAN ON UNCONFINED SMALL MAMMAL POPULATIONS IN SOUTHERN ALBERTA

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FIELD EXPOSURE AND BIOLOGICAL EFFECTS OF GRAIN FARMERS DURING CARBOFURAN APPLICATION FOR GRASSHOPPER CONTROL

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EXECUTIVE SUMMARY

Sampling and Analysis: Eight farmers in Southern Alberta were selected as exposure subjects and were monitored for exposure to carbofuran (Furadan 480F) and subsequent intoxification while they were engaged in the routine operation of mixing, loading and spraying of insecticide to control grasshoppers. One jug of Furadan 480F contains four litres of concentrate at a concentration of 480 grams of active ingredient per litre. This is mixed with water and applied at a rate of 160 g active ingredient per hectare. Each exposure subject was supplied with a set of personal protective equipment consisting of a new clean cover-all made of perforated and spun-bonded polyethylene textile over his ordinary work clothing, work gloves made of heavy duty PVC material, and a disposable respirator with cartridge specified for pesticide vapor.

A series of exposure studies were conducted in June and July of 1986, employing six (6) assistants and summer students. Personal exposure to the insecticide was determined by newly established methods employing:

1. a polyurethane foam plug for the inhalation route;
2. twenty-three (23) membrane patches for the dermal route;
3. pre- and post-exposure urine and blood samples for biological monitoring.

The total number of analytical samples amounted to 400, and analytical methods used were high pressure liquid chromatography with fluorescence spectrometer for the active ingredient and degradation products and UV-VIS spectrophotometer for blood enzymes.

Route of Exposure: Pesticide can enter the human body by occupational exposure through three routes: inhalation, dermal, and ingestion. The exposure control strategy may be established depending on the major route of entry. One subject was exposed to the insecticide to a level of 379 μg/kg of insecticide applied. A total of 57.5% of the insecticide mass was deposited on the wrists and hands, while 42% of the total mass was found on the rest of the body surface such as face, shoulders and legs. The remaining fraction of 0.5% by mass would have been inhaled. Thus the major route of exposure to carbofuran was found to be the dermal route, or through the skin; however, carbofuran is not absorbed through the skin.

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Dermal Exposure: The dermal dose of the farmer who was exposed at 379 µg/kg of insecticide applied may be calculated as follows, as a worst case. The recommended rate of application for grasshopper control was 160 g active ingredient per hectare, which was equivalent to mixing 1 jug (1920 g) in one tankful and spraying over 12 hectares. If he were to spray a maximum of six (6) tankfuls in a day, he would be exposed to 4366 µg of carbofuran, and if his body weight is 85 kg, then he would be exposed to 51.4 µg/kg body weight/day. The dermal LD_{50} of carbofuran or the amount required to kill 50% of test animals (rats) through dermal application is 6,800,000 µg/kg BW. Thus the safety margin in a worst case situation between the total amount that the farmer would be exposed to (51.4 µg/kg BW) and the lethal dose of carbofuran (6,800,000 µg/kg BW) would be 100,000. This figure shows that even in the worst case situation, the personal protective equipment provided performed satisfactorily.

Inhalation Exposure: If a farmer with 85 kg bodyweight were to spray a minimum of six (6) jugs in a day, he would be exposed to 0.27 µg/kg BW by the inhalation route. The four (4) hour inhalation LC_{50} (inhalation lethal dose) of carbofuran for rats is 133 µg/litre of air. The farmer performing medium level work inhales 29.3 litres of air per minute. If the inhalation lethal dose for the rat was assumed to be 11,000 µg/kg BW for four (4) hours to succumb, the safety margin in a worst case situation between the amount actually inhaled (0.27 µg/kg BW) and the inhalation lethal dose (11,000 µg/kg BW) would be exceeding 40,000. This figure demonstrates that the personal protective equipment (respirator) provided performed satisfactorily.

Safety Margin: The safety margins derived above the dermal and inhalation exposures were based on the LD_{50} and LC_{50} values. Actually, the safety margins should have been based on the no-effect exposure level of the chemical, in which case, more realistic values would have been obtained. Unfortunately, however, the no-effect exposure level is not available at present. The prescribed occupational exposure limit (OEL) set under the provincial chemical hazard regulation is based on the 8-hour time-weighted average (TWA) concentration. The inhalation exposure in the worst case situation remained far below the OEL.

Dermal Exposure. The dermal dose of the farmer who was exposed at 100 g/ha of insecticide applied can be calculated as follows, as a worst case. The recommended rate of application for grasshopper control was 100 g active ingredient per hectare, which was equivalent to mixing 1 kg (1.0 L) in one tankful and spraying over 10 hectares. If he were to spray a maximum of six (6) tankfuls in a day, he would be exposed to 7200 µg of carbofuran, and if his body weight is 65 kg, then he would be exposed to 110.8 µg/kg body weight/day. The dermal LD_{50} of carbofuran or the amount required to kill 50% of test animals (rats) through dermal application is 2,500,000 µg/kg BW. Then the safety margin in a worst case situation between the total amount that the farmer would be exposed to (110.8 µg/kg BW) and the lethal dose of carbofuran (2,500,000 µg/kg BW) would be 22,563. This figure shows that even in the worst case situation, the personal protective equipment provided performed satisfactorily.

Inhalation Exposure. If a farmer with 65 kg bodyweight were to spray a maximum of six (6) tanks in a day, he would be exposed to 0.37 µg/kg BW by the inhalation route. The four (4) hour inhalation LC_{50} (inhalation lethal conc. of carbofuran) for rats is 0.1 mg/litre of air. The farmer performing medium level work inhales 29.4 litres of air per minute. If the inhalation lethal dose for the rat was assumed to be 17,000 µg/kg BW for four (4) hours of exposure, the safety margin in a worst case situation between the amount actually inhaled (0.37 µg/kg BW) and the inhalation lethal dose (17,000 µg/kg BW) would be exceeding 40,000. This figure demonstrates that the personal protective equipment (respirator) provided performed satisfactorily.

Safety Margin. The safety margins derived above for the dermal and inhalation exposures were based on the LD_{50} and LC_{50} values. Actually, the safety margins should have been based on the no-effect exposure level of the chemical. In which case, more realistic values would have been obtained. Unfortunately, however, the no-effect exposure level is not available at present. The threshold occupational exposure limit (TLV) set under the provincial chemical hazard regulation is based on the 8-hour time-weighted average (TWA) concentration. The inhalation exposure of the spray crew during the operation remained far below the TLV.

Urinary Exposure: Only seven percent (7%) of the amount of carbofuran to which farmers were exposed was excreted in the urine. This amounted to 28 µg of active ingredient/kg of carbofuran applied. An extremely low but still detectable amount of hydroxycarbofuran was found in the urine of one exposed subject only.

Blood Enzymes: Exposure to carbofuran is known to cause the inhibition of blood enzymes (acetyl and pseudo cholinesterases) and subsequent depression of the central nervous system (CNS). There was no relationship between blood cholinesterase levels and the amount of carbofuran sprayed, the amount to which the farmers were exposed, and the amount detected in the urine. The concentrations of cholinesterases in blood remained within the normal ranges for healthy male adults. No detectable amount of carbofuran was found in the ambient air in the field during the busiest spraying period of grasshopper control.

Health Effects: No exposure subject experienced any symptoms of carbofuran poisoning due to continuous exposure periods ranging from 8 to 300 minutes (5 hours) per day. The biological monitoring of all exposed subjects continued for four (4) consecutive days following exposure. A period of five (5) hours at a time would be almost the maximum length that a normal healthy farmer would spray per day and could be considered the worst case situation. Most farmers did not have any reason to spray carbofuran for two (2) or more consecutive days for long hours per day. Usually, they sprayed for one long day every one or two weeks. In one case, however, a farmer sprayed 50% of a jug (0.96 kg) each day for two consecutive days and then about 75% of a jug (1.6 kg) each day for the next two consecutive days. He, too, experienced no symptoms of carbofuran poisoning.

Exposure Control: The amount of carbofuran exposure by farmers who used a truck-mounted sprayer was seven (7) times the exposure encountered by farmers who used tractors without cabs or cab-mounted tractors. The amount of carbofuran that penetrated was 20% of the carbofuran deposited on the surface of the coveralls.

Biological Effects: The state of health of participating farmers, including liver and kidney functions, were examined by analyzing the following blood counts: blood cell types, hemoglobin, electrolytes, creatinine, glucose, albumin, and various blood enzymes such as alkaline phosphatase, creatine phosphokinase, lactate dehydrogenase. The results indicated that analytical data were within normal ranges and showed that all the farmers were healthy throughout the study period and that no adverse health effects were induced by the exposure to carbofuran.

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INTRODUCTION

The grasshopper outbreak in Southern Alberta for the past three years was the most severe infestation experienced within the last ten years. Consequently, farmers were required to spray highly toxic insecticides several times over the season to control the infestation. In this unusual situation, exposure to insecticides among farmers through various routes of entry would have been considerably high if proper devices of personal protection are not used. However, no exposure monitoring was conducted to determine what levels of insecticides farmers might have contacted to and what the acute health consequences of such exposures might have to them.

The grasshopper infestation was anticipated to be as severe or even worse than the year 1985. The insecticides will be used consequently in much greater quantities resulting in potentially higher exposure among farmers. A survey by Alberta Agriculture (1983) revealed that in a year when the amount of spraying might be considered average, five (5) percent of Alberta farmers experienced acute symptoms of insecticide poisoning. Such symptoms include: stomach cramps, nausea, diarrhea and convulsions.

A series of field exposure studies were conducted in the summer of 1986 in two farming communities of Southern Alberta with the following objectives:

1) To determine the total amount of the highly toxic insecticide, carbofuran, a farmer may be exposed to after spraying for extended periods of day for consecutive days;
2) To determine if such an amount of carbofuran is high enough to cause significant blood enzyme depression, changes in liver function, which metabolizes the chemical, or overt symptoms of poisoning;

3) To determine if there is any correlation between the amount of carbofuran exposed to, the amount of blood enzymes inhibited, and any changes in liver function; and finally,
4) To determine to what extent the farmers' exposure is reduced by personal protective equipment recommended and used by farmers.

During the June-July period of 1986 in Southern Alberta, eight (8) farmers were selected as exposure subjects and were monitored for occupational exposure to carbofuran (Furadan 480F) and subsequent intoxification while they were engaged in the routine operation of mixing, loading and spraying of insecticide to control the stage of grasshopper infestation. Each exposure subject was supplied with a set of personal protection equipment (PPE) specified by the design of this study. The personal exposure measurement was made by newly developed methods employing a foam plug for inhalation, membrane patches for dermal, and pre- and post-exposure urine and blood samples for biological monitoring. The total number of analytical samples amounted to 400. The results of laboratory analyses revealed critical parameters in personal exposure; they are, the major route of entry in such working environments, the safety margins for the dermal and inhalation doses of carbofuran, urinary excretion and metabolite, the inhibition of blood enzymes and, finally, the maximum allowable exposure limit of carbofuran that did not affect human health. The results would provide the regulatory agency to re-examine other critical parameters in applicator exposure control, that is, the maximum amount of insecticide to be applied at a time operation.

1. Toxicity of Carbofuran

a) Animal Toxicity

Technical formulation of carbofuran is an odorless, white crystalline solid with a molecular weight of 221.26. The chemical name is 2,3-Dehydro-2,2dimethyl-7-benzofuranyl methylcarbamate. The melting point is between 150 and 152°C and has a vapour pressure of 2×10^{-5} mmg Hg at 33°C. Its solubility at 25°C in water is slight, being 700 ppm. Carbofuran is known to be a reversible cholinesterase (ChE) inhibitor. Cholinesterase is an enzyme essential to the transmission of nerve impulses (Palmer et al., 1973).

The oral LD_{50} of carbofuran is 8.7 and 8.0 mg/Kg in male and female rats, respectively. Dermal dose at 1000 mg/Kg caused no deaths and only minimal symptoms in rats of both sexes. Thus, although the oral toxicity of carbofuran is considerably high, it has a dermal toxicity of a low order of magnitude. The acute oral median lethal dose in the form of suspended liquid (wettable powder) is 23.4 mg/Kg and the acute dermal LD_{50} is 1350 mg/Kg in male rats. Acute ocular studies with 25% wettable powder(WP) and 75 WP in rats reveal LD_{50} values of 21.5 mg/Kg and 18.0 mg/Kg, respectively.

The inhalation toxicity of carbofuran is also relatively high. The LC_{50} of the 50% wettable powder (WP) was 108 and 133 mg/m^3 for male and female rats, respectively. Seventy-five percent WP had a respiratory LC_{50} to guinea pigs (mixed sexes) of 53 mg/m^3. In all concentration levels equivalent to 0.56 mg/m^3 of carbofuran 75% WP, no cholinesterase depression was noted in the Rhesus monkey following a six-hour exposure. Slight but significant cholinesterase depression occurred at 0.86 mg/m^3. As with other carbamate-type anticholinesterases, there is a very short time lag between exposure and onset of symptoms for carbofuran.

Carbofuran may, therefore, be classified in terms of lethal dosages as highly toxic chemical through oral and inhalation routes, and moderately toxic chemical through dermal routes.

In a chronic feeding study in rats, the highest dietary level having no effect was 25 ppm; for the dog 20 ppm (Tobin, 1970). Dietary levels of 50 ppm produced significant inhibition of plasma, erythrocyte and brain cholinesterase levels.

b) Human Toxicity

Carbamates, in general, undergo metabolic reactions in vivo involving oxidation, reduction, hydrolysis and conjugation. In mammals, the liver is the primary organ involved in these reactions where the responsible enzymes are associated with the smooth endoplasmic reticulum of liver cells.

Although it was once assumed that hydrolysis of the ester linkage would be one of the most important metabolic reactions of carbamate because of the wide distribution of estrases in body tissues, it has been shown that there is considerable variation in rates of hydrolysis of different carbamate compounds and different species, and also that the non-hydrolytic process can at times be significant. It was found that the in vivo rate of hydrolysis of both N-hydroxymethyl carbofuran and 3- ketocarbofuran in the rat was faster than for the parent compound. However, at present, the nature of the enzymes responsible for carbamate hydrolysis in vivo is not fully understood.

Carbamates have also been shown to undergo conjugation reactions. One of the mechanisms by which carbofuran can be eliminated from the body involves conversion of the parent compound to metabolites which are, in turn, converted to water-soluble conjugates and then excreted in the urine and the bile.

Conjugation can, therefore, be an important step in the detoxification of carbofuran in the body.

The reference ranges of blood enzymes are known as: pseudocholinesterase in the serum of an adult (3.0 - 8.0 KU/L) and acetylcholinesterase in the whole blood of a male adult (6.5 - 11.0 KU/L). However, the sign of enzyme inhibition is not a strict indicator of exposure to cholinesterase inhibitors (Wolfsie, 1952).

c) Symptoms

In general, carbamate insecticides are rated as moderately toxic as compared to other insecticides of mild to extremely toxic (organophosphorous) and mild to highly toxic (chlorinated hydrocarbons), according to the classification by teh National Institute of Occupational Safety and Health (U.S.A.).

The pharmacologic action is anticholinesterase with symptoms of CNS depression manifested by constriction of pupils, salivation, profuse sweating, lassitude, muscle incoordination, nausea, vomiting, diarrhea, epigastric pain and tightness of chest. In treatment of carbofuran poisoning, the speed is imperative and atropine injection and oxygen respiration is standard.

d) Carcinogenicity

Studies in the mid 70's reported significant occurrences of tumors in test animals exposed to carbofuran; however, the studies had certain limitations. The statistical considerations, the high mortality and the insufficient data on the histopathological examinations on the initial studies indicated that the studies were not diagnostic of carbofuran's carcinogenic potential. Further testings of carbofuran have provided an adequate data base for evaluating the carcinogenic potential of carbofuran.

e) Mutagenicity

Carbofuran was assessed for mutagenic activity in various strains of bacteria by means of the induced liver metabolic-activating system in rats. Carbofuran per se was reportedly negative with respect to mutagenic activity in this test system. When carbofuran was fed to mice together with $NaNO_2$, no evidence of mutagenic effects was detected employing the mouse micronuclei test.

2. Description of Field Application

a) Description of Study Subject

i) Pre-test Medical History

In late May, eleven (11) volunteer farmers residing in the districts of Hussar and Fort Macleod were approached by a team of clinical investigators for pre-test health examination. The medical history of each volunteer was taken by administering a series of standard questionnaires and pre-test blood samples were collected. Out of eleven (11) subjects, eight (8) farmers were selected as the study subjects, among whom seven (7) being exposure subjects and one (1) as a control subject. The age of exposure subjects ranged from 21 to 58 or a mean age of 41. The blood samples were analyzed at the Department of Laboratory Medicine, Foothills Hospital for general health aspects of subjects and for the baseline value of pseudocholinesterase. Splitted samples of blood were also sent to Dr. S. Hanson Associates for baseline value of acetylcholinesterase. After the eight (8) farmers were selected, they signed a form of informed consent provided through an approval of the Informed Consent Subcommittee of the Conjoint Committee on the Medical Ethics of The University of Calgary, Faculty of Medicine and affiliated teaching institutions (Appendix). At the end of the study, a short summary of the

study was sent to each of the volunteer farmers according to the signed consent.

ii) Personal Protective Equipment (PPE)

Under certain circumstances, it is impractical to eliminate a hazard of health risk by engineering control of emission, change in procedures, mechanical safeguards, limiting exposure time to hazardous substances, or other administrative controls. Personal protective equipment (PPE) becomes the worker's last resort in self-defense.

The PPE used in the study include: coveralls, underwear, a respirator and a pair of gloves. All exposure subjects used their own cap and boots. The use of safety glasses or eye goggles was not recommended. The coveralls used was perforated Tyvek R by E.I. Dupont Co., made of spun-bonded polyethylene textile. Tyvek is water repellent and the perforation provided breathable advantage due to its permeability. After each test period, a consensus was obtained among wearers of Tyvek coveralls about comfort of the material (Williams, 1980; Dedek, 1980). The participants' attitude toward the use of protective equipment was closely observed while they were equipped with devices by trained field crew (DeJonge et al., 1984). Gloves used were classified under chemical hazard use and made of polyvinyl chloride (PVC)/nitrile and provided the good insulation without sacrificing the flexibility of glove material (Williams, 1979). The respirator used was classified under the pesticide and organic vapour use. Each time, a new respiraetor was supplied to each exposure subject immediately prior to the exposure period by breaking the seal. The re-use of such a respirator for later spray application was discouraged. All exposure subjects were provided with clean new underwear (cotton T-shirt) to be worn immediately after the dermal patches were adhered to body surface.

b) Description of Field Work

The control of grasshopper infestation was intended at the infant stage; therefore, the target area included the roadside ditches in addition to the regular fields. Among seven (7) farmers, six (6) of them used tractor-drawn sprayers only or pick-up mounted sprayers only. Only one farmer reported that he used both types of sprayers. The boom size for pick-up sprayers was 10-12 m long; however, the field sprayers used wider booms of 20-24 m long. Only one tractor had a protective cab mounted; therefore, the ratio of enclosed versus natural ventilation provided to operators is 4 (enclosed) to 2 (natural) (Table 1 and 2, Appendix).

Since the formulation used was known to create less vapour component, the spray operation was made even under windy conditions with a certain degree of spray drift. The wind speed during the test periods ranged from 6.5 to 27 Km/hr. The air temperature for the test period remained at the 15-31°C level and the relative humidity tended to remain lower during the mid-days (28-30%) and to rise toward late evenings (45%) (Table 3).

The formulation used is flowable liquid and diluted with water and uniformly mixed. The formulation "flowable" has lower vapour pressure (non-volatile) and does not produce a vapour component when properly mixed with a carrier liquid (water). The spray additive used by two farmers was pH-2 (pH reducing agent by Daid Agr. Services Ltd.) which contained sulphuric acid and corrosive. A jug of pH-2 (1 litre) was mixed with 300 gallons (1363 litres) of water, reducing the pH values from 8.0 to 6.0 (Table 8).

3. Sampling and Analytical Methods

a) Dermal Patch

Materials used as patch surfaces are: a transparent dressing "Tegaderm" (membrane film by 3M, 60 x 75 mm) and a similar material for cannula fixation "Applica" (by BDF, 60 x 75 mm). Both films are backed with adhesive; therefore, prior to mounting, the films were attached together with the adhesive side in. A mounting tape used is 75 m x 45 m adhesive support bandage "Elastoplast". A window of 33 x 33 mm was cut providing 10 cm^2 of surface area. Before use, patches were kept in a clean container and the exposure side of the membrane was covered with a protective film of paraffin.

There were, in total, 20 patches attached to an exposure subject (Figure 1). Among them, four were located outside of the coveralls for the determination of the protection factor of clothing. Each time, a hand wash sample was taken immediately after the exposure period. A plastic bag of 1 litre capacity was filled with 300 ml of clinical grade isopropyl alcohol and both hands just below the wrist were washed in the bag.

Exposed patches were solvent-extracted and the active ingredient (carbofuran) was quantified by using HPLC (Perkin Elmer, Model 650-10M) with fluorescence spectrometer. The detection limit of the instrument is 0.1 ppm (Appendix, Details of Analytical Method). A typical method of determining the dermal toxicity of a pesticide was discussed earlier. The method adopted for laboratory experiments of rats is exactly opposite. A known amount of a pesticide was applied on a patch and the skin contact was made (Noakes, 1969). Photochemical degradation is one of the dissipation routes to be considered in evaluating carbofuran residues. It was reported that when carbofuran was absorbed on a silt loam and exposed to a light at 26°C, the half life determined was 13.6 days (Palmere, 1977). The period of photolysis during the

field sampling ranged less than one hour to six hours; therefore, the photochemical degradation during these short periods would have been negligible.

b) Determination of Body Surface Area

The total surface area of the human adult (male) is predicted from the equation of DuBois:

$$(\text{Surface Area, m}^3) = (\text{Body Weight, Kg})^{0.425} \times (\text{Height, m})^{0.725} \times 0.07184$$

Details of the measurement of anthropometric variables are discussed elsewhere (Harris, 1978). Values of total body surface were readily obtained from a nomogram for determination of body surface on page 537 of Geigy's Scientific Tables (7th Edition, 1975). A common value for a male adult of average size is known to be approximately two. The values obtained ranged from 1.86 (J.Z.) to 2.18 m^2 (L.N. and H.R. (Appendix).

The total surface area of a body may be proportionated by anatomical body regions (Spear et al., 1977) and values of regional surface were used in dermal exposure assessment (Franklin et al., 1981) (Appendix). There were 18 anatomical regions on which patches were to be located.

c) Inhalation Sample

The airborne vapour and droplets of carbofuran were captured by using an inhalation sampler of the polyurethane foam principle. The collection efficiency of the foam plug for other pesticides has been known (Billings, 1980). A plug of polyurethane foam with equivalent density to furniture cushion (20 mm diameter x 75 mm long) was pre-rinsed with laboratory grade ethanol and then installed in a glass tube with a bulb. The drying tube

(Fisher catalog 09-215A) is made of pyrex glass and is 15 mm inside diameter and 100 mm long. A neck of drying tube (6 mm outside diameter) was connected to a portable pump which provided the suction at a rate of 5 litre/minute. The time of initiating and terminating the pump operation were recorded to obtain the mean concentration during the sampling period. An identical sampler as a background was aspirated during the same sampling period at a site far away from pesticide operation. Drying tubes were stored, having the inlet sealed with a paraffin film and, immediately after the exposure period, sealed again and stored in a cooler for transport. Aspirated foam plugs were solvent-extracted in the laboratory and the active ingredient (carbofuran) was quantified by using HPLC (Appendix, Details of Analytical Method).

d) Urine Sample

The daily void of urine was collected by each of exposure and control subjects in a standard container (Fisher catalog No. 14-375-115). The container is made of collapsible polyethylene and intended for 24-hour collection. The container is amber tinted and graduated from 500 to 3500 cc.

Urine samples were collected in a sequence; one day immediately before the test exposure, one day immediately after the test exposure, and post-test exposure period of four days. Sample containers were kept solid, frozen in a cold storage (dark, -14°C) until transported to the analytical laboratory. Active ingredients detected in the urine samples are: carbofuran, 3-hydroxy carbofuran and 3-keto carbofuran. The recovery (%) of each ingredient was also determined in the laboratory (Appendix, Details of Analytical Method).

e) Blood Sample

Blood samples were collected for both exposure and control subjects during the pre-test exposure and post-test exposure periods. In order to examine the normal values of cholinesterases for individual study subjects, blood samples were taken at the time of interview for commitment. At this time (May 28 and 29, 1986), all collaborating subjects signed the form of informed consent which was approved by the Subcommittee on Informed Consent of the Conjoint Medical Ethics Committee of The University of Calgary, the Faculty of Medicine and affiliated institutions. Blood samples were collected for all exposure subjects immediately before and after the test-exposure period and then for every 24 hours for four consecutive days.

A third year student in the degree nursing course was specially trained for two weeks in the skill of vein puncture procedures at the Department of Laboratory Medicine, Foothills Hospital. The blood sampling method employed is a standard vein puncture with the "Vacutainer system".

For pseudocholinesterase, vacutainer tubes used are 10 ml capacity with red stopper by Becton Dickinson attached with disposable multi-sample needle of gauge 22 by Venojet. For acetylcholinesterase, 5 ml of whole blood was collected in vacutainer tubes of green stopper by Becton Dickinson attached with a disposal multi-sample needle of gauge 22 by Venojet. All apparatus for blood sampling were supplied by the Department of Laboratory Medicine, Foothills Hospital. Blood samples collected for pseudocholinesterase and other blood tests were sent to the Foothills Hospital for analysis. An instrument used is UV-VIS spectrophotometer (Perkin-Elmer Lambda 3B/e). Blood samples for acetylcholinesterase were analyzed by Dr. S. Hanson and Associates (Laboratory Medicine) in Edmonton. The principle of enzyme analysis is the standard method of B.C. Workers' Compensation Board, No. 2200 issued on October 19, 1984 (Appendix, Details of Analytical Method).

4. Results and Discussion

a) Operational and Environmental Conditions

Eight (8) farmers were monitored in this study. Some relevant information is presented about each one and the volume of work done during the monitoring period. The average age was 41 years (range 21-58), average weight was 84 kilograms (range 71-104), and average height was 1.78 metres. The number of jugs of Furadan used per farmer ranged from one-half to six (6), the application rate ranged from three-quarters to twice the recommended amount, and the length of operation which was monitored ranged from 8 minutes to 300 minutes. Two (2) farmers used pH-2 as a spray additive to reduce the rate of carbofuran hydrolysis in alkaline water (Table 1).

Listed is the type of work done by each individual and the type of spraying equipment used during the operation. Three (3) farmers used three-quarters to one-ton pick-up trucks. Two (2) of them were monitored while they mixed their chemical in the sprayer and sprayed the entire load. The third individual mixed his chemical earlier during the day and was monitored only while he was spraying. Another three (3) farmers used tractor sprayers to spray their chemical. Two (2) of these mixed and sprayed while the third individual sprayed only. One farmer had a tractor with a cab. The seventh farmer carried out one mixing operation only while the eighth farmer was used as a control subject (Table 2).

The farmers who used a pick-up truck to spray kept their windows closed during the operation. The farmer who used a tractor with a cab also worked with his cab closed and air-conditioning turned off.

The environmental conditions prevailing at the time of monitoring are as follows. Conditions varied from sunny to scattered clouds to cloudy, and windspeed was in the range of 6.5 to 25 kilometers per hour. Temperatures ranged from 15 to 31°C and relative humidity from 28-45% (Table 3).

b) Dermal Exposure

The body surface area of each subject was determined from his weight (Kg) and height (m) according to a nomogram in Geigy's Scientific Tables (1975) (Appendix).

Total body surface area was then proportionated into body regions according to Spear et al. (1977) (Appendix). The surface area of 17 regions of the body of each farmer was determined. Based upon the amount (μg) of carbofuran found on the dermal patch located at each region and the surface area of that region, a concentration was calculated (Appendix). The concentration on the hands and wrists was determined from the amount on the wrist patches and in the hand wash. Based on the total amount (Kg) of carbofuran sprayed by each farmer, the values were normalized (μg per body region/Kg carbofuran sprayed) (Table 4 and Appendix).

The total number of dermal patches administered to seven (7) exposure subjects was 175 excluding blanks and photolysis samples. Practically, the rate of patch recovery after exposure was close to 100%. Some of the hand patch samples were replaced by handwash samples. All of the ankle patches were located on the left side only. The high rate of recovery should raise the quality assurance of data as far as the dermal exposure is concerned. Usually, the recovery in this size of exposure measurement remains at the 85% level. The use of "Tegaderm" patches proved its efficiency in this study.

It is known that the highest dermal deposition takes place on the wrist region. The ratio of the wrists/the forearms was 18.5 for L.N. and 10 for H.M. For all other subjects, the ratio to reference region remained at unity.

The patches on head-neck regions were exposed to the airborne pesticide or to accidental wipe by contaminated surfaces (hands). The degree of contamination may be due to personal habit or by spray drift. Only values

obtained for L.N. showed extremely high. The value calculated is 2.65 and remained higher than other subjects. The degree of pesticide contamination in the calf region may be affected by the type of boots each subject wore at the time of exposure study and also by the nature of work done. The deposition on the left calf of M.G., who only mixed and loaded carbofuran, was remarkably high as compared to M.H. who only sprayed (ND). Another extreme value found in L.N. in the right calf may be explained due to contaminated boots while preparing the spray liquid.

The total dermal exposure of the body, wrists and hands to carbofuran (μg) per Kg of active ingredient sprayed is summarized (Table 5). The results suggested that there was practically no difference in total body exposure between the two (2) farmers who mixed and sprayed with pick-up trucks (values 144 and 343.6) compared to the one who sprayed only (value = 201). Consequently, all results for these three individuals were grouped together and treated statistically as "truck sprayers". Similarly, there appeared to be no difference in total body exposure between individuals who mixed and sprayed with a cabless tractor or a cab tractor and the one who sprayed only with a cabless tractor. Results on these three individuals were treated as "tractor sprayers".

Average dermal exposure of the total body and the wrists and hands, respectively were 162 μg/Kg and 218 μg/Kg a.i. sprayed by the six farmers. Average total body exposure of the three truck sprayers was 230 μg/Kg of active ingredient sprayed which was about 2.7 times more than the average (86 μg/Kg) to which tractor sprayers were exposed. Average wrist and hand exposure of the truck sprayers was 342 μg/Kg compared to 30 μg/Kg a.i. sprayed for tractor sprayers, the difference being 11 times more for truck sprayers.

This higher exposure of truck sprayers could be caused by entry of the chemical into the cab and slow exit to the outside.

Of the six (6) subjects monitored during the spraying operation, one (L.Z.) had an unusually high amount (5510 µg/Kg) of the chemical on his wrists and hands. This value was not included in the calculation of the average exposure for tractor sprayers.

The 17 body regions were compressed to four (excluding wrists and hands) and the dermal deposition on these regions calculated for truck and tractor sprayers (Table 6). The results indicated that only the arms were equally exposed to carbofuran for these two (2) groups. For the upper body parts, head and lower body parts, the truck sprayers were exposed to 8, 4 and 2 times more chemical than tractor sprayers. Overall average distribution of the chemical to these four (4) major body parts was 21% to the face, 27% to the upper body, 38% to the lower body, and 14% to the arms. Therefore, the chemical was fairly evenly distributed throughout the whole body surface except the arms which were exposed to less (Table 7).

Results in the single individual (G.M.) who mixed one jug of Furadan (1.92 Kg) only are shown (Table 4). The chemical was deposited only on the shoulders, right thigh and left calf. This type of distribution was expected for a mixer. His total body was exposed to 152 µg of chemical/Kg mixed, while his wrists and hands were exposed to 41 µg/Kg a.i. mixed. When these values were compared to the other six (6) farmers who mixed and/or sprayed, the total body exposure was about the same (162 µg/Kg a.i. for the six farmers), but the wrist and hand exposure was lower (218 µg/Kg for the six farmers). This comparison suggested that if someone mixed only, his body would be exposed to a certain amount of chemical. If this same individual were to spray, his body would probably pick up some more chemical but would also be retained in the

clothing. After several hours of spraying, the amount of chemical deposited on the skin would, therefore, depend on a number of factors including absorption by clothing textile. Consequently, there may not be major differences in dermal deposition of pesticide between someone who mixed a concentrate and someone who mixed and sprayed for several hours.

c) Protection Factor of Work Clothing

Protection factors (PF) were determined at shoulder, chest and right forearm. Values of PF were calculated by dividing the deposition density of active ingredient on the outside patch by the density of the inside patch. For seven (7) exposure subjects, only nine (9) PF values were obtained due to the fact that active ingredient on only 15 out of 21 patches were detectable for outside and that 10 out of 21 were detectable for inside patches. The PF values ranged from 34 to 100%. The values for non-detectable inside patches may be regarded as infinite; however, those subjects with lower PF values (1.0-2.0) may require special attention. Since all subjects were supplied with clean coveralls and underwear, the lower values of PF may simply be an indicator of attitudes towards personal hygiene (Table 8).

d) Inhalation Exposure

The concentration of carbofuran (µ/L) in the air in the immediate vicinity of the breathing zone, the amount (µg) assumed to have been inhaled by each farmer, and the normalized values per kilogram of active ingredient sprayed (µg/Kg) are shown (Table 9). The results indicated that a farmer could be exposed to an average of 2 µg of carbofuran for every Kg active ingredient sprayed (Appendix). If truck sprayers were compared to tractor sprayers, the farmer using a truck would be exposed to 5.5 times more chemical

than the farmer using a tractor. As shown previously, body exposure for truck sprayers was also about 3 times higher than for tractor sprayers. Exposure of the hands and wrists was 11 times more for truck sprayers than for tractor sprayers. Since all farmers wore a respirator during the operation, exposure through the inhalation route would be zero.

e) Major Route of Entry

The values of active ingredient for body weight and exact exposure period for individual subject were compared between dermal and inhalation exposures. The common sense fact is that for non-vapour producing formulation, the dermal exposure is usually predominant and that the inhalation exposure is not significantly high. While the dermal exposure may be controlled by clean work clothing and practices, the inhalation exposure can only be controlled by employing efficient respirators.

The total amount of carbofuran to which a farmer could be exposed (assuming that he wore no respirator) during the mixing and spraying operation is shown (Table 10). The results indicated that the average total exposure was 379 µg of a.i. per Kg of pesticide applied. Of this, the torso region of the body was exposed to 159 µg, the wrists and hands to 218 µg and another 2 µg was inhaled. The values correspond to 42%, 57.5% and 0.5% for the body, wrists and hands, and inhalation, respectively. Therefore, about 99.5% of total exposure occurred to the skin; only 0.5% was inhaled. If truck sprayers were compared to tractor sprayers for total exposure, the former would be nearly 7 times more exposed than the latter. Another study indicates that the total exposure of mixer was approximately 10 times that of the tractor operator when carbofuran was sprayed with a tractor-drawn field sprayer (Nagy, 1986).

f) Urinary Excretion

The amount of carbofuran sprayed and the amount recovered in the urine over a four (4) day post-exposure period were determined (Table 11). All farmers who mixed and sprayed excreted some carbofuran in the urine.

However, the daily excretion was not consistent. On some days, no carbofuran was found but was again detected the very next day. This inconsistency may be dependent on the detection level of the analytical instrument. The method used for carbofuran analysis allowed detection of 0.1 µg/L in the urine. Most of the levels observed in the samples were just above or below this detection limit.

The one farmer (G.M.), who mixed only for about 8 minutes, had no carbofuran residue in his urine. Another farmer (R.L.) was used as a control subject to check for background carbofuran levels in urine. None was found over a four-day period.

The percentage of carbofuran found in th urine based on the calculated total exposure level was determined (Table 11). The average concentration excreted in the urine was about 28 µg a.i./Kg sprayed. This amounted to about 7% of the actual exposure level (Table 12).

Hydroxy carbofuran, a metabolite of carbofuran, was observed in one farmer (R.H.) only. The level was about 8 µg a.i./Kg sprayed or 1.5% of the actual exposure level. No attempt was made in this study to identify or quantify keto-carbofuran, another metabolite, or other bound metabolites in the urine.

g) Cholinesterase Inhibitor

The percentage activity of acetylcholinesterase (AchE) in whole blood and of pseudocholinesterase (ChE) in plasma was determined (Table 13). The AchE

values were within a narrow range, from 89-107%. The ChE values for three exposure subjects (N.L., M.H. and G.M.) had a narrower range (94-105%), whereas the values for two (2) exposure (R.H. and H.M.) and one control (R.L.) subjects had a much wider range (79-130%). These results suggest that there is no relationship between cholinesterase activity and the amount of carbofuran sprayed, amount to which the farmers are exposed, and amount detected in the urine. The activity of acetylcholinesterase and pseudocholinesterase in whole blood and plasma was presented (Appendix).

h) Ambient Concentration of Carbofuran

Air samples were collected at six (6) farmsteads from one-half hour to five (5) hours in duration during the height of the grasshopper spraying season in Southern Alberta. No detectable concentrations of carbofuran were found. This result suggested that even though a significant amount of spraying had taken place in the vicinity, the chemical did not volatilize or remain in the air at a concentration high enough to be detected.

i) Effects on Liver and Kidney Function

More than 30 blood parameters were clinically measured to determine the effects of carbofuran on the blood and on liver and kidney function. These parameters include red cell count, total white cell count, hemoglobin, hematocrit, mean cell volume, counts of lymphocytes, monocytes and granulocytes as well as electrolytes, creatine, glucose, total protein, albumin, cholestrol and four (4) enzymes such as alkaline phosphatase, creatine kinase, lactic dehydrogenase and aspartic transaminase. Results indicated that all the farmers were healthy and that no effects occurred to the liver and kidney.

j) In Perspective

The results in this study indicate that an adult farmer is exposed to a total of 379 μg of carbofuran per kilogram of the chemical applied. A jug of Furadan 480F contains 4 litres at 480 g a.i. per litre which amounts to 1920 gm. The recommended rate is 160 gm/ha which is equivalent to mixing 1 jug or 1920 gm of 1365 litres (300 gallons) and spraying it over 12 hectares (30 acres). If a farmer were to spray one tank of Furadan, he would be exposed to about 728 μg of carbofuran. If he weighs an average of 85 Kg (190 lbs.), then he would be exposed to about 8.5 μg/Kg per tank. The oral LD_{50} of carbofuran is 11 mg/Kg and the dermal LD_{50} is 6800 mg/Kg. A farmer who mixes and sprays the chemical would be exposed mostly through the dermal route. Even if the oral LD_{50} value is taken, the safety margin between actual human exposure and animal toxicity would be more than a thousand-fold.

The results also indicate that of the total amount of carbofuran that a farmer may be exposed to, 99.5% is dermal and only 0.5% is inhalation. This low inhalation amount should also add to the safety of the chemical.

This study further shows that coveralls used in this study reduced about 65% of dermal exposure, that only 7% of the amount of chemical exposed to (and most likely entered the body) is excreted, and that no cholinesterase inhibition occurred.

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Table 1: Some relevant information about each farmer and the volume of work carried out during the monitoring period

Subject	Age (y)	Body Wt (Kg)	Height (m)	Jugs(a) A.I. Used	Appl Rate (g/ha)	A.I. Used (Kg)	Area Sprayed (ha)	Length of Operation (min)	Spray Additive Used
RH	38	91	1.89	2	320(b)	3.84	12	100	pH-2
NL	49	104	1.75	3	120	5.76	48	257	
HM	58	88	1.78	0.5	160	0.96	6	34	
JV	54	78.5	1.75	1.5	240	2.88	12	105	
MH	37	71	1.84	6	160	11.52	72	300	pH-2
LZ	47	79	1.65	1	120	1.92	16	115	
GM	21	79	1.78	1	-	1.92	-	8	
RLC	21	79	1.75	(control subject)		-	-	-	-
AVG	41	83.7	1.78						

(a) One jug of Lunadan 480F contains 4 litres at 480 g a.i./L

(b) Recommended rate = 160 g a.i./hectare

Table 2: Type of work done and spraying equipment used by each of the seven farmers who mixed and/or sprayed

Subject	Type of Operator	Type of Sprayer	Boom Width (m)	Cab
RH	Mixed and sprayed	Pick-up Truck	12	Yes
NL	Mixed and sprayed	Pick-up Truck	12	Yes
HM	Sprayed	Pick-up Truck	10	Yes
JV	Mixed and sprayed	Tractor	24	No
MH	Mixed and sprayed	Tractor	20	Yes
LZ	Sprayed	Tractor	24	No
GM	Mixed	-	-	-

Table 3: Environmental conditions during the mixing and spraying operations

Subject	Type of Operation	Wind Speed (Km/h)	Air Temp (°C)	Relative Humidity (%)	Weather Conditions
RH	Mixed and sprayed	25	21	31	Cloudy
NL	Mixed and sprayed	11	23	30	Sunny
HM	Sprayed	6.5	20.5	45	Cloudy
JV	Mixed and sprayed	11	31	28	Scattered Clouds
MH	Mixed and sprayed	8	25	39.5	Scattered Clouds
LZ	Sprayed	27	15	34	Sunny
GM	Mixed	6.5	20.5	45	Cloudy

Table 4: Normalized values for the distribution of carbofuran for body region/Kg (CO) of active ingredient sprayed (μg/Kg)

	Subject	RH	NL	HM	JV	MH	LZ	GM
Body Regions	Amount Sprayed (Kg)	3.84	5.76	0.96	2.88	11.52	1.92	1.92(c)
Face		15.9	95.3	30.2	9.4	2.34	13.5	0
Head(a)		0	16.0	0	0	0	13.5	0
Neck		0	3.5	0	0	0	0	0
Shoulders		19.3	25.7	0	0	17.1	0	35.0
Chest		0	15.1	85.4	0	13.4	0	0
Back		0	0	85.4	0	0	0	0
R-Upper Arm		0	9.0	0	0	0	23.4	0
L-Upper Arm		0	9.0	0	0	0	23.4	0
R-Forearm		9.6	0	0	0	2.9	16.6	0
L-Forearm		9.6	25.7	0	11.5	0	0	0
Hips		0	17.2	0	30.6	0	0	0
R-Thigh		25.5	34.0	0	0	7.6	43.8	46.4
L-Thigh		25.5	17.0	0	0	0	43.8	0
R-Calf		19.3	38.5	0	0	0	0	0
L-Calf		19.3	12.8	0	0	5.7	0	70.3
Ankles		0	24.7	0	0	5.5	0	0
Subtotal (μg/Kg)		144.0	343.6	201.0	51.4	54.4	178	151.6
Wrist(b) and Hands		126.8	613.9	286.5	23.0	37.6	5510.4	41.1
Total (μg/Kg)		270.8	957.5	487.5	74.3	92.0	5688.5	192.7

(a) Side and back of head

(b) Wrist patches and handwashes

(c) Amount mixed

Table 5: Total dermal exposure of the body, wrists and hands to carbofuran (μg/Kg) of active ingredient sprayed

Type of Equipment	Pick-up Truck			Tractor		
Type of Operator	Mixed & Sprayed	Mixed & Sprayed	Sprayed	Mixed & Sprayed	Mixed & Sprayed	Sprayed
Subject	RH	NL	HM	JV	MH	LZ
Body Region						
Total Body (minus wrists and hands)	144.0(a)	343.6	201.0	51.4(b)	54.4	178
Wrists and hands	126.8(a)	613.9	286.5	23.0(d)	37.6	5510.4(e)

Overall average = 162 μg/Kg for total body and 218 μg/Kg for wrists and hands.

(a) Average for truck sprayers = 230 μg/Kg a.i. sprayed

(b) Average for tractor sprayers = 86 μg/Kg a.i. sprayed

(c) Average = 342 μg/Kg for truck sprayers

(d) Average = 30 μg/Kg for tractor sprayers

(e) This value was too high and was not included in calculation of the average for tractor sprayers

Table 6: Dermal deposition of carbofuran to four major body regions of truck sprayers and tractor sprayers

Body Region	Truck	Tractor	Factor Difference
Head - Neck	53.6(a)	12.8	4.2 x
Shoulders to Wrist	77.0	10.2	7.5 x
Arms (excluding wrists and hands)	21.0	25.9	1.2 x
Hips to Ankles	77.9	45.6	1.7 x

Figures derived from Appendix 5.

(a) Values are in μg of chemical/Kg sprayed

Table 7: Percentage distribution of carbofuran to the four major body regions of the six farmers who mixed and sprayed

Major Body Region	% Distribution of Carbofuran
Head to Neck	21
Shoulders to Waist	27
Arms	14
Hips to Ankles	38

Table 8: Degree of protection provided by coverall and ordinary work clothes at three body regions: shoulders, chest and forearm

Body Region	Shoulders			Chest			Forearm		
Subject	Over Coverall(a) (μg/Kg)	On Skin(b) (μg/Kg)	%(c) P	Over Coverall (μg/Kg)	On Skin (μg/Kg)	% P	Over Coverall (μg/Kg)	On Skin (μg/Kg)	% P
RH	148	74	50	87	0	100	74	37	50
NL	222	148	34	1046	87	92	37	0	100
HM	70	0	100	82	82	0	0	0	-
JV	65	0	100	0	0	-	0	0	-
MH	131	197	0	232	154	34	164	33	80
LZ	0	0	-	74	0	100	63	32	49
GM	67	67	0	79	0	100	34	0	100

(a)(b) Concentration of carbofuran (μg/Kg a.i. sprayed or mixed) per body region.

(c) Percentage protection provided by clothing and is calculated as 100-(ratio of value on skin ÷ value on coverall x 100). If pesticide is detected on the coverall but not on the skin, protection is considered to be 100%. If more pesticide is detected on the skin than on the coverall, protection is considered to be zero.

Table 9: Amount of carbofuran (μg/Kg a.i.)(a) assumed to have been inhaled by the farmers

Subject	Conc'n in Air (μg/L)	Amount A.I. Sprayed (Kg)	Amount A.I. Inhaled (μg)	Amount A.I.(a) Inhaled/Kg (μg/Kg)
RH	.0014	3.84	4.1	1.1
NL	.0033	5.76	24.8	4.3
HM	.0042	0.96	4.2	4.4
JV	0	2.88	0	0
MH	.0024	11.52	21.1	1.8
LZ	0	1.92	0	0
GM	0	1.92	0	0

(a) μg/Kg = μg of a.i. inhaled/Kg sprayed

(b) Average for truck sprayers = 3.3 μg/Kg a.i.

(c) Average for tractor sprayers = 0.6 μg/Kg a.i.

(d) Overall average = 1.9 μg/Kg a.i.

Table 10: Total amount of carbofuran(a) to which a farmer could be exposed during a mixing and spraying operation

Source of Exposure	Truck			Tractor			Avg	Avg (%)
	Subject			Subject				
	RH	NL	HM	JV	MH	LZ(b)		
Total Body	144.0	343.6	201.0	51.4	54.4	178	158.8	42.0
Wrists and Hands	126.8	613.9	286.5	23.0	37.6	5510.4	217.6	57.5
Inhalation	1.1	4.3	4.4	0	1.8	0	2.3	0.5
Total	271.9(c)	961.8	491.9	74.4(d)	93.8	5688.4	378.7	100

(a) Values in μg/Kg a.i. sprayed

(b) Values for LZ not included in calculation of averages

(c) Average for truck sprayers 575.2 μg/Kg a.i.

(d) Average for tractor sprayers 84.1 μg/Kg a.i.

Table 11: Amount of carbofuran (µg) excreted in the urine within a four-day post-exposure period in farmers

	RH(a)		NL		HM(b)		MH		LZ		GM			
Sprays Post-Exposure	A.I. Sprayed (Kg)	Amt in Urine (µg)	A.I. Sprayed (Kg)	Amt in Urine (µg)	A.I. Sprayed (Kg)	Amt in Urine (µg)	A.I. Sprayed (Kg)	A.I. in Urine (µg)	A.I. Sprayed (Kg)	A.I. in Urine (µg)	A.I. Sprayed (Kg)	A.I. in Urine (µg)	A.I. Sprayed (Kg)	A.I. in Urine (µg)
0	7.68	0	5.76	-	0.96	0	11.52	60	1.92	50	1.92	-		
1	-	50	-	25	0.96	130	-	65	-	25	-	0		
2	-	60	-	0	1.6	-	-	50	-	50	-	0		
3	-	50	-	25	1.6	0	-	0	-	-	-	0		
4	-	-	-	0	-	90	-	65	-	-	-	0		
Total		160		50		220		240		125		0		

(a) RH sprayed two loads that day but was monitored only for one.

(b) HM sprayed 1.92 Kg and 1.2 Kg a.i. two and five days before day of monitoring.

Table 12: Percentage of carbofuran found in urine based on calculated total exposure level

Subject	A.I. Sprayed (Kg)	Total Amt in Urine (μg)	Conc'n(a) in Urine (μg/Kg)	Actual Exposure (μg/Kg)	%(b)
RH	7.68	160	20.8	543.8(e)	3.8
NL	5.76	50	8.7	961.8	0.9
HM	0.96(c)	120	23.4	491.9	4.8
FV	2.88	-(d)	-	-	-
MH	11.52(d)	240	20.8	93.8	22.2
LZ	1.92	125	65.0	5688.4	1.1
Average			27.7		6.6

(a) μg/Kg = average of carbofuran by Kg a.i. sprayed

(b) % = concentration (μg/Kg) of a.i. in urine as a paercentage of total actual exposure

(c) Amounts sprayed in days 1 and 5 pre-exposure not used in calculation

(d) Urine samples not collected

(e) Value adjusted to include the one additional load sprayed the same day

Table 13: Percentage activity of acetylcholinesterase (AchE) and pseudocholinesterase (ChE) in farmers exposed to carbofuran

	RH		NL		HM		MH		GM[a]		RL[b]	
DAYS	AchE	ChE	AchE	ChE	AchE	ChE	AcheE	ChE	AchE	ChE	AchE	ChE
	-	100					100	100				
	100	120	100	100	100	100	100	101	100	100		
	101	120	96	102	95	93	103	99	100	96	100	100
	97	119	107	105	89	94	101	103	90	97	-	
	100	130	92	99	91	79	99	97	93	98	98	109
	95	128	99	100	94	97	95	95	97	102	98	118
	103	96	96	96	96	99	95	95	94	94	99	116
	95	101	-	-	97	97	97	97	91	96	89	98

(a) GM mixed one jug or 1.92 Kg a.i. only

(b) RL was control subject

6. Appendices

Details of equipment used by each farmer during the spraying operation

Approval by the Conjoint Medical Ethics Committee of the Faculty of Medicine, The University of Calgary, and the Affiliated Teaching Institutions

Informed Consent Form

Pesticide Questionnaire Form

Body surface area of farmers determined from nomogram

Surface area of 17 regions of the body calculated

Material Safety Data Sheet, Carbofuran, Flowable
Guide to Protection in Alberta, Furadan
EPA Chemical Profile, CAS 1563-66-2, Carbofuran

Amount of carbofuran on the surface of 17 body regions
Dermal distribution of carbofuran
Concentration of carbofuran in the breathing zone
Analytical Method No. 2200, Cholinesterase in whole blood and plasma
Acetyl cholinesterase activity in farmers
Pseudocholinesterase activity in farmers
Percentage activity of acetylcholinesterase and pseudocholinesterase

Appendix 1: Details of equipment used by each farmer during the spraying operation

Subject	Type of Equipment	Model of Equipment	Model of Sprayer	No. of Nozzles	Nozzle Type	Pressure (kPa)	Travelling Speed (km/h)
RH	Truck	3/4 ton	JD 335	25	LF 2-80	280	17.6
NL	Truck	Ford 1 ton	JD 335	25	8004	125	12
HM	Truck	Dodge 1 ton	JD 335	17	L2-80	280	12.8
JV	Tractor	Case		43	8002 LP	100	12.8
MH	Tractor	John Deere	Vertee VS 2300		2008 SS	180	14.8
LZ	Tractor	John Deere		20	TK-SS	280	12.8

Appendix 2: Body surface area of farmers determined from nomogram

Subject	Age (y)	Weight (Kg)	Height (m)	Total Body Surface Area (cm^2)
RH	38	91	1.89	21800
NL	49	104	1.75	21800
HM	58	88	1.78	20600
JV	54	78.5	1.75	19400
MH	37	71	1.84	19300
LZ	47	79	1.65	18600
GM	21	79	1.78	19800

Appendix 3: Surface area (cm^2) of 17 regions of the body calculated according to Spear et al. (1977)

Body Region	% of Total	Subject						
		RH	NL	HM	JV	MH	LZ	GM
Face	2.8	610.4	610.4	576.8	543.2	540.4	520.8	554.4
Head(a)	2.8	610.4	610.4	576.8	543.2	540.4	520.8	554.4
Neck	1.2	261.6	261.6	247.2	232.8	231.6	223.2	237.6
Shoulders	6.8	1482.4	1482.4	1400.8	1319.2	1312.4	1264.8	1346.4
Chest	8.0	1744	1744	1648	1552	1544	1488	1584
Back	8.0	1744	1744	1648	1552	1544	1488	1584
R-Upper Arm	4.8	1046.4	1046.4	988.8	931.2	926.4	892.8	950.4
L-Upper Arm	4.8	1046.4	1046.4	988.8	931.2	926.4	892.8	950.4
R-Forearm	3.4	741.2	741.2	700.4	659.6	656.2	632.4	673.2
L-Forearm	3.4	741.2	741.2	700.4	659.6	656.2	632.4	673.2
Hips	9.1	1983.8	1883.8	1874.6	1765.4	1756.3	1692.6	1801.8
R-Thigh	9.0	1962	1962	1854	1746	1737	1674	1782
L-Thigh	9.0	1962	1962	1854	1746	1737	1674	1782
R-Calf	6.8	1482.4	1482.4	1400.8	1319.2	1312.4	1264.8	1346.4
L-Calf	6.8	1482.4	1482.4	1400.8	1319.2	1312.4	1264.8	1346.4
Ankles	6.5	1417	1417	1339	1261	1254.5	1209	1287
Wrists & Hands	6.8	1482.4	1482.4	1400.8	1319.2	1312.4	1264.8	1346.4
Total Body Surface Area	100	21800	21800	20600	19400	19300	18600	19800

(a) Side and back of head

Appendix 4: Amount of carbofuran (μg) on the surface area of 17 body regions.

Body Region	Subject						
	RH	NL	HM	JV	MH	LZ	GM
Face	61	549	29	27	27	26	0
Head^(a)	0	92	0	0	0	26	0
Neck	0	20	0	0	0	0	0
Shoulders	74	148	0	0	197	0	67
Chest	0	87	82	0	154	0	0
Back	0	0	82	0	0	0	0
R-Upper Arm	0	52	0	0	0	45	0
L-Upper Arm	0	52	0	0	0	45	0
R-Forearm	37	0	0	0	33	32	0
L-Forearm	37	148	0	33	0	0	0
Hips	0	99	0	88	0	0	0
R-Thigh	98	196	0	0	87	84	89
L-Thigh	98	98	0	0	0	84	0
R-Calf	74	222	0	0	0	0	0
L-Calf	74	74	0	0	66	0	135
Ankles	0	142	0	0	63	0	0
Subtotal (μg)	553	1979	193	148	627	342	291
Wrists & Hands^(b)	487	3536	275	66	433	10580	79
Total (μg)	1040	5515	460	214	1060	10922	370

(a) Side and back of head

(b) Amount calculated as sum on two wrist patches plus handwash.

Appendix 5: Dermal distribution of carbofuran (µg/Kg as sprayed) to 17 body regions of truck sprayers and tractor sprayers

Body Region	Avg for 3 Truck Sprayers	Avg for Major Body Region	Avg for 3 Tractor Sprayers	Avg for Major Body Region
Face	47.1[(a)]	Head	8.3	Head
Head	5.3	53.6	4.5	12.8
Neck	1.2		0	
Shoulders	15.0	Upper Body	5.7	Upper Body
Chest	33.5		4.5	
Back	28.5	77.0	0	10.2
R. Upper Arm	3.0	Arms	7.8	Arms
L. Upper Arm	3.0		7.8	
R. Forearm	3.2	21.0	6.5	25.9
L. Forearm	11.8		3.8	
Hips	5.7	Lower Body	10.2	Lower Body
R. Thigh	19.8		17.1	
L. Thigh	14.2		14.6	
R. Calf	19.3	77.9	0	
L. Calf	10.7		1.9	45.6
Ankles	8.2		1.8	

(a) Values are in µ a.i./Kg sprayed

Appendix 6: Calculations showing the concentration of carbofuran in the breathing zone and the amount assumed to be inhaled

Subject	Amount on PUF (μg)	Pump Rate (1/min)	Time (min) Exposed	Conc'n in Breathing Zone (μg)	Amount[a] Inhaled (Kg)	Amt of A.I. Sprayed (Kg)	Amt of A.I. Inhaled/Kg Sprayed
RH	0.5	3.5	100	.0014	4.1	3.84	1.1
NL	3.0	3.5	257	.0033	24.8	5.76	4.3
HM	0.5	3.5	34	.0042	4.2	0.96	4.4
JV	0	3.5	105	0	0	2.88	0
MH	2.5	3.5	300	.0024	21.1	11.52	1.83
LZ	0	3.5	115	0	0	1.92	0
GM	0	3.5	8	0	0	1.92	0

(a) The inhalation rate of an adult man performing medium level activity is assumed to be 29.3 1/min (1.76 m^3/1) (Spector, 1956).

Appendix 7: Acetylcholinesterase (AchE) activity in farmers exposed to carbofuran

		RH			NL			HM		
		A.I. Sprayed (Kg)	AchE Km/L	% Activity	A.I. Sprayed	AchE	% Activity	A.I. Sprayed	AchE	% Activity
PRE-EXP	8					8.4	100		9.2	100
	7									
	6									
	5							1.2		
	4									
	3									
	2							1.92		
	1		8.6	100						
DAY	PRE	7.68	8.7	101	5.76	8.1	96	0.96	8.7	95
	POST		8.3	97		9.0	107	0.96	8.2	89
POST-EXP	1		8.6	100		7.7	92	1.6	8.4	91
	2		8.2	95		8.3	99	1.6	8.6	94
	3		8.9	103		8.1	96		8.8	96
	4		8.2	95					8.9	97

Appendix 8: Acetylcholinesterase (AchE) activity in farmers exposed to carbofuran

		MH			GM			RL		
		A.I. Sprayed (Kg)	AchE Km/L	% Activity	A.I. Sprayed	AchE	% Activity	A.I. Sprayed	AchE	% Activity
PRE-EXP	8		7.8	100		8.4	100			
	7									
	6									
	5							1.2		
	4									
	3									
	2							1.92		
	1		7.8	100						
TEST DAY	PRE	11.52	8.0	103		8.9	100	Control Subject	8.4	100
	POST		7.9	101		8.0	90		8.2	89
POST-EXP	1		7.7	99		8.3	93		8.2	98
	2		7.4	95		8.6	97		8.2	98
	3		7.4	95		8.4	94		8.3	99
	4		7.6	97		8.1	91		7.5	89

Appendix 9: Pseudocholinesterase (ChE) activity in farmers exposed to carbofuran

		RH			NL			HM		
		A.I. Sprayed (Kg)	ChE Km/L	% Activity	A.I. Sprayed	ChE	% Activity	A.I. Sprayed	ChE	% Activity
PRE-EXP	8		16.2	100		13.2	100		18.5	100
	7									
	6									
	5							1.2		
	4									
	3									
	2							1.92		
	1		19.5	120						
DAY	PRE	7.68	19.5	120	5.76	13.4	102	0.96	17.2	93
	POST		19.3	119		13.9	105	0.96	17.4	94
POST-EXP	1		21.1	130		13.1	99	1.6	14.6	79
	2		20.7	128		13.2	100	1.6	18.0	97
	3		15.6	96		12.6	96		18.4	99
	4		16.3	101					18.0	97

Appendix 10: Pseudocholinesterase (ChE) activity in farmers exposed to carbofuran

		MH			GM			RL		
		A.I. Sprayed (Kg)	ChE Km/L	% Activity	A.I. Sprayed	ChE	% Activity	A.I. Sprayed	ChE	% Activity
PRE-EXP	8		11.6	100		20.2	100			
	7									
	6									
	5									
	4									
	3									
	2									
	1		11.7	101						
DAY	PRE	11.52	11.5	99	1.92	19.4	96		19.0	100
	POST		11.9	103		19.6	97			
POST-EXP	1		11.2	97		19.8	98		20.8	109
	2		11.0	95		20.6	102		22.4	118
	3		11.0	95		19.0	94		22.0	116
	4		11.2	97		19.4	96		18.8	99

THE
UNIVERSITY
OF CALGARY

Health Sciences Centre
3330 Hospital Drive N.W., Calgary, Alberta, Canada T2N 4N1

Faculty of MEDICINE
DEAN'S OFFICE

Telephone (403) 220-6842

1986-06-27

Dr. K. Yoshida
Department of Community Health Sciences
The University of Calgary
Calgary, Alberta

Dear Dr. Yoshida:

Re: Monitor Field Exposure and Biological Effects of Grain Farmers During Carbofuran Application for Grasshopper Control

The above-named proposal has been reviewed by the Conjoint Medical Ethics Committee of the Faculty of Medicine, The University of Calgary, and the Affiliated Teaching Institutions.

I am happy to report that the protocol has been approved from an ethical point of view.

Please accept the Committee's best wishes for success in your research.

Yours sincerely,

T. D. Kinsella, M.D.
Assistant Dean (Medical Bioethics) and
Chairman, Conjoint Medical Ethics Committee

TDK:smh

c.c. Ethics Reviewers
R & D Committee
Dr. E. J. Love (information)

Olympic Village and Speedskating - 1988

THE
UNIVERSITY
OF CALGARY
Health Sciences Centre
3330 Hospital Drive N.W., Calgary, Alberta, Canada T2N 4N1

Faculty of MEDICINE
DEAN'S OFFICE

Telephone (403) 220-6842

1986-06-02

Dr. K. Yoshida
Department of Community Health Sciences
Faculty of Medicine
The University of Calgary
Calgary, Alberta

Dear Dr. Yoshida:

Re: Monitor Field Exposure and Biological Effects of Grain Farmers During Carbofuran Application for Grasshopped Control

As Chairman of the Conjoint Medical Ethics Committee of the Faculty of Medicine, University of Calgary, and the Affiliated Teaching Institutions, I am writing to advise you that the above-noted study has been granted interim approval with respect to ethical considerations, subject to the revised consent form .

Please note that interim approval permits you to initiate your study but that the latter must not deviate from the approved protocol.

Your protocol will be submitted to the Conjoint Medical Ethics Committee for final review. Upon completion of the latter process, you will be advised of any relevant recommendations.

Yours sincerely,

S. Murray-Hicks

for T. D. Kinsella, M.D.
Assistant Dean (Medical Bioethics),
and Chairman, Conjoint Medical
Ethics Committee

TDK:smh

c.c. R & D Committee
Dr. E. J. Love (information)

Olympic Village and Speedskating - 1988

Copy

THE
UNIVERSITY
OF CALGARY

Faculty of MEDICINE
Department of COMMUNITY HEALTH SCIENCES

3330 Hospital Drive N.W., Calgary, Alberta, Canada T2N 4N1

Telephone (403) 220-4285/87/88

1986-03-17

Dr. T.D. Kinsella, Chairman
Conjoint Medical Ethics Committee
Faculty of Medicine
The University of Calgary
Calgary, Alberta T2N 4N1

Dear Dr. Kinsella:

Re: Monitor Field Exposure and Biological Effects of Grain Farmers During Carbofuran Application for Grasshopper Control

As per the attached letter from Dr. M. Hussain of Alberta Agriculture, the proposed program initially submitted to the Future for Farm Research Grant was approved by the Environment Council of Alberta.

I am a co-investigator of this project and will be responsible for the selection and health protection of 22 study subjects. I am submitting a copy of the research program for review by the Conjoint Medical Ethics Committee.

Since the program will be initiated on April 1, 1986 and a series of field exposure measurements is expected to start in May, your prompt response will be greatly appreciated.

Sincerely yours,

Ken Yoshida, Ph.D.
Visiting Associate Professor
Department of Community Health Sciences

KY/sg
Attachment
cc: Dr. E.J. Love

Olympic Village and Speedskating - 1988

THE
UNIVERSITY
OF CALGARY

Faculty of MEDICINE
Department of COMMUNITY HEALTH SCIENCES

3330 Hospital Drive N.W., Calgary, Alberta, Canada T2N 4N1

Telephone (403) 220-4285/87/88

SUBJECT CONSENT FORM

1. Research Project:	Monitoring Field Exposure and Biological Effects of Grain Farmers During Carbofuran Application for Grasshopper Control
2. Investigator:	Dr. K. Yoshida, Department of Community Health Sciences, Faculty of Medicine, The University of Calgary, 3330 Hospital Dr. N.W., Calgary, Alberta T2N 4N1, phone 220-4297

In consenting to participate in this study, I understand that I will be acting as an exposure/control subject under the research program explained by the investigators. I also understand that medical information about me which is connected with this test will become available to the investigators at The University of Calgary, as well as to the medical staff administering these tests. In all other respects, all information relating to me will be kept strictly confidential.

I understand that I am under no obligation to participate in this study and that my decision to participate or not will have no influence on the quality of my medical care. I am free to withdraw from the study at any time.

Having understood the above conditions, I agree to participate in this study.

Participant's Signature (Name) ____________________ (Date)

Alberta
AGRICULTURE
Plant Industry Division

Alberta Agriculture, Plant Industry Division, J.G. O'Donoghue Building, 7000 - 113 Street, Edmonton, Alberta, Canada T6H 5T6

April 14, 1986

Dear

Thank you for agreeing to help us out in the study to measure farmer's exposure to Furadan.

I enclose a summary of the project which you can review.

You will be required to help in the following ways:

1. When you are ready to spray Furadan, you will give us a call the day before.
2. On the day of the spraying, we will come down to your farm and give you a disposable coverall to wear. The coverall will have cloth patches inside and outside to collect spray droplets.
3. You will also be given a small air pump which will be hooked on to your belt. The pump is very small and light and will not get in your way. The pump will collect air from your surroundings.
4. You will be given urine bottles to collect all urine up to 3 days after you have stopped spraying.
5. You will then go out and spray.
6. When you have finished spraying, we will take the pump and coveralls, and a small sample of blood from your arm. We will take these to the lab for tests.
7. At this time, your job is finished. We will come around only to pick up the urine samples and take one more small blood sample.

.../2

Please remember that if you are not planning to use Furadan this year, you will not qualify for the study. But, you should not go out and buy Furadan just to be in the study.

I am arranging a meeting with all farmers who will be taking part in the study on April 30, 1986. At this meeting we will discuss in detail what will be done. I will give you a telephone call to confirm.

Your assistance on this project is really appreciated.

Yours sincerely,

M. Hussain
Pesticides Coordinator

Tel. 427-4438

MH/du
A/041486/L03
Encl.

FURADAN PROJECT

The grasshopper outbreak in southern Alberta last summer was the most severe infestation ever experienced within the last ten years. Consequently, farmers were required to spray some extremely toxic insecticides several times over the season to control the pests. In this unusual situation exposure to fumes, spills and splashes, and spray drift would have been high. However, no monitoring was done to determine what levels of pesticides farmers might have been exposed to and what the acute health consequences of such exposures might have been to them.

This year, the grasshopper infestation is anticipated to be as severe or even worse than last summer. The insecticides carbofuran, carbaryl, deltamethrin, and dimethoate will be used again in perhaps much greater quantities than last year. Farmer exposure is anticipated to be high again.

A survey by Alberta Agriculture (1983) revealed that in a year when the amount of spraying might be considered average, five percent of farmers experienced acute symptoms of poisoning. Such symptoms include stomach cramps, nausea, diarrhea, and convulsions. In an unusual year such as is anticipated for 1986 where the number of sprays applied can be many, the number of poisoned individuals can be much higher. An individual who has become ill from such symptoms can be debilitated for several days or weeks resulting in loss of valuable working time.

The objectives of this study are to determine:

i) the total amount of highly toxic insecticide, carbofuran (Furadan), a farmer may be exposed to after spraying several hours per day;

ii) if such an amount of this insecticide is high enough to cause significant cholinesterase depression, changes in liver function which metabolizes the chemical, or symptoms of poisoning;

iii) if there is any correlation between the amount of carbofuran exposed to, the amount of cholinesterase inhibited, and any changes in liver function;

iv) to what extent full safety clothing and equipment worn by the applicator during pesticide mixing and spraying reduces exposure.

The insecticide carbofuran is chosen in this study because it is very toxic orally (LD50=8mg/kg) and is used the most by applicators due to its high efficacy and low cost.

STUDY DESIGN, METHODOLOGY AND ANALYSES

1. Selection of Exposure and Control Subjects: A group of 20 male farmers in southern Alberta will be selected as exposure subjects. The selected farmers will represent a cross section of the general population base for farmers. They will be pre-examined by an occupational health physician. Two control subjects will be selected from the general population.

2. Medical Ethics: The proposed program will be submitted to the medical ethics committee of the University of Calgary for review and approval. Prior to the selection of study subjects, a meeting will be held with them to comply with the standard practice of informed consent (toxicity, health effects, and personal protection aspects).

3. Health Examination at Pre and Post Exposure Periods: The physician will examine the subjects for physical fitness, medical histories including daily dietary habits, smoking and drinking status, type and name of medication, and chemicals and other pesticides used during the test period. A health questionnaire to be used has been approved by Health and Welfare Canada. After exposure, a 14 day record of changes in behaviour and ill-health will be recorded by the subject and reviewed by the physician. In the event of any untoward problems, the subjects would be required to seek the physician's advice.

4. Sampling Method:

i) Direct Method of Determination:

(a) Dermal Exposure: Each exposure subject will be supplied with a clean coverall, cap, and underwear immediately prior to the start of the spraying operation. Protective devices such as eye goggles, respirator with organic cartridges, and rubber gloves will also be provided. Cellulose patches will be located at 14 anatomical regions and will be affixed inside and outside each subject's work clothing. The selection of anatomical locations is according to the guidelines of Health and Welfare Canada. All patches will be collected after exposure and stored in a cooler. This method has been well established.

(b) Inhalation Exposure: The principle of personal sampling within the breathing zone of exposure subjects will be employed by using a polyurethane foam plug that will be aspired for the entire period of the spraying operation. The exposed plugs will be stored in a cooler. The technique has been well established.

(c) Ambient Air Quality: A stationary sampler of polyurethane foam plug will be located at the mixing-loading area in order to obtain the background concentration of active ingredient in the ambient air. During the sampling period, the meteorological observation of air temperature and humidity, wind speed and direction will be made by using hand-held instruments.

Indirect Method of Exposure Determination:

(a) Urinary Excretion: Daily (cumulative) urine samples will be collected by all study subjects for the pre and post-exposure period up to 3 days after the end of exposure. Urine samples will be stored in brown bottles in a cooler. Each bottle will be date-marked and the total daily void will be registered in the laboratory by a nurse. Samples will be divided into two parts--one for active ingredient analysis by the Food Lab

of Alberta Agriculture and another for creatinine and liver function by Foothills Hospital.

(b) Blood samples: Blood samples will be collected from all study subjects by employing a standard method of vein puncture with the vacutainer system. Samples will be collected once before spraying, once immediately after spraying, and 24 hours after spraying. The blood samples will be analyzed for creatinine, liver function, red cell and plasma cholinesterases.

5. Analytical Methods: Exposed cellulose patches, polyurethane plugs and part of urine samples will be extracted and mass-quantified for active ingredient by a GC at Alberta Agriculture Food Lab in Edmonton. The analyses of blood and urine samples for red cell and plasma cholinesterases, creatinine, and liver function will be done at the Department of Pathology Clinical Laboratory at Foothills Hospital by standardized methods.

PESTICIDE QUESTIONNAIRE

NO. ______

I. IDENTIFICATION

1. Name ______________________
2. Address ____________________
3. Postal Code ________________
4. Telephone __________________

5. Age ____ 6. Sex ____ 7. Weight ____ 8. Height _____

9. Education: (highest level achieved)

____

II. HISTORY OF PESTICIDE EXPOSURE

10. How many years have you been working with pesticides? __________

11. Which chemicals have you used within the last six months? (January-June 1986)

Chemical	Formulation Type
Avadex	______________
Treflan	______________
Vitavax (straight without Lindane)	______________
Vitavax (dual with Lindane)	______________
Other Seed Treatment Chemical(s)	______________
Furadan (granular)	______________
Counter (granular)	______________
Decis (cutworms)	______________
Lorsban (cutworms)	______________
Others ______________________	
______________________	

12. Which chemicals did you use last fall? (August-November 1985)

Chemical	Formulation
______________	__________
______________	__________
______________	__________
______________	__________
______________	__________
______________	__________

13. Have you worked with pesticides in the following manner (yes/no):
i) Mixing.................................... _____
ii) Ground application........................ _____
iii) Pilot - aerial application................ _____
iv) Flagman - aerial application.............. _____
v) Formulating............................... _____
vi) Manufacturing............................. _____
vii) Other (specify) ______________________________
____
____

14. In the past 6 months while working with pesticides how often did you wear:
(Place either 1, 2, 3 or 4 in the space provided)
1 = always   3 = sometimes
2 = usually   4 = never

i) Coverall................................ _____
Fabric type...............................
ii) Gloves.................................. _____
Type of material..........................
iii) Goggles................................. _____
Vented...........; Unvented............
iv) Respirator.............................. _____
Type.....................................
v) Hat..................................... _____
Type.....................................
vi) Boots................................... _____
Type.....................................
vii) Apron................................... _____
Type.....................................

15. In the past 12 months, while working with pesticides, did you work in a protective cab or a tractor?
Yes _______   No _______

16. In the past 12 months, when working with pesticides, did you:
i) Change over-clothing after using pesticides Yes ____ No ____
ii) Change under-clothing after using pesticides Yes ____ No ____
iii) Shower after using pesticides Yes ____ No ____
iv) Wash hands after mixing pesticides Yes ____ No ____
v) Wash hands and face before eating Yes ____ No ____
vi) Wash pesticide-contaminated clothes the same day Yes ____ No ____
vii) Wash pesticide-contaminated clothes with other clothing Yes ____ No ____
viii) Smoke on the job Yes ____ No ____
ix) Drink alcohol on the job Yes ____ No ____

17. In the past 12 months, have you noticed any of the following symptoms:

Answer in the box beside each 1, 2 or 3:
1. yes
2. no
3. don't know

a. Central Nervous System
i) weakness ______
ii) dizziness ______
iii) headache ______
iv) insomnia ______
v) tremors ______
vi) muscle spasms or cramps ______
vii) impaired memory ______

b. Gastro-Intestinal System
i) Nasea, vomiting ______
ii) diarhea ______
iii) blood in stools ______
iv) abdominal cramps ______
v) loss of appetite ______
vi) weight loss ______

c. Respiratory System
i) chest pain or tightness ______
ii) shortness of breath ______
iii) cough ______
iv) sputum production ______

d. Eyes
i) irritation, burning ______
ii) redness ______
iii) blurring of vision ______
iv) double vision ______

e. Skin
i) irritation, dryness ______
ii) rashes or skin staining ______
iii) chemical burns ______

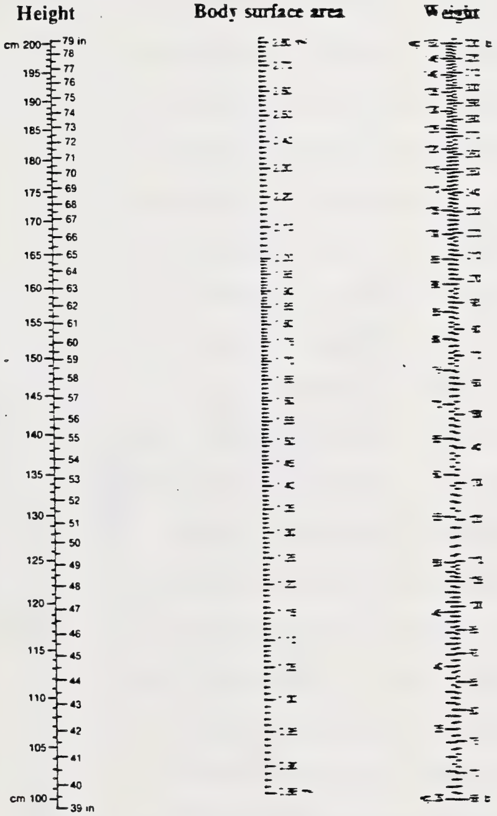
f. Exocrine System
i) increased sweating ______
ii) increased salivation ______
iii) increased tearing ______

g. Urogenital System
i) increased urination ______
ii) loss of urine involuntarily ______

h. Do you have any of the following
i) heart disease ______
if "yes" specify ____________________
ii) lung disease ______
if "yes" specify ____________________
iii) liver disease ______
if "yes" specify ____________________
iv) kidney disease ______
if "yes" specify ____________________
v) cancer ______
if "yes" specify ____________________
vi) a history of allergies ______
if "yes" specify ____________________
vii) Other unusual symptoms (please list)
____
____
____

Table B-1 Body Surface Area of Adults

Nomogram for determination of body surface area from height and weight.* A straightedge placed from the patient's height (left column) to his weight (right column) will give his body surface area (middle column).



From Documenta Geigy scientific tables, ed. 7, courtesy CIBA-Geigy Limited, Basel, Switzerland
From the formula of Du Bois and Du Bois, Arch. Intern. Med. 17:863, 1916. $S = W^{0.425} \times H^{0.725} \times 71.84$, or $\log S = \log W \times 0.425 + \log H \times 0.725 + 1.8564$ (S = body surface in cm², W = weight in kg, H = height in cm)

* *(Source: Freireich, 1966, 219–244)*

Use the following formula to convert adult dosage in mg/kg to mg/m²:
mg/kg × 37 = mg/M²

Table B-2. Conversion Tables to Determine Body Surface Area (In Kilograms and Centimeters)

Body surface area (m^2) as a function of height (cm) and weight (kg)*

Height (cm)	Weight (kg)																							
	4	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	**26**	**28**	**30**	**32**	**34**	**36**	**38**	**40**	**44**	**48**	**52**	**56**	**60***
50	0.25	0.31	0.36	0.40	0.44	0.48	0.51	0.54	0.57	0.60	0.63	0.66	0.68	0.71	0.73	0.75	0.78	0.80	0.82	0.86	0.90	0.94	0.97	1.01
55	0.26	0.32	0.37	0.42	0.46	0.50	0.53	0.57	0.60	0.63	0.66	0.68	0.71	0.74	0.76	0.78	0.81	0.83	0.85	0.90	0.94	0.98	1.01	1.05
60	0.27	0.33	0.39	0.43	0.48	0.52	0.55	0.59	0.62	0.65	0.68	0.71	0.74	0.76	0.79	0.81	0.84	0.86	0.88	0.93	0.97	1.01	1.05	1.09
65	0.28	0.34	0.40	0.45	0.49	0.53	0.57	0.61	0.64	0.67	0.70	0.73	0.76	0.79	0.82	0.84	0.87	0.89	0.91	0.96	1.00	1.05	1.09	1.13
70	0.29	0.36	0.41	0.46	0.51	0.55	0.59	0.63	0.66	0.69	0.73	0.76	0.79	0.81	0.84	0.87	0.89	0.92	0.94	0.99	1.04	1.08	1.12	1.16
75	0.30	0.37	0.42	0.48	0.52	0.57	0.61	0.64	0.68	0.71	0.75	0.78	0.81	0.84	0.87	0.89	0.92	0.95	0.97	1.02	1.07	1.11	1.16	1.20
80	0.31	0.38	0.44	0.49	0.54	0.58	0.62	0.66	0.70	0.73	0.77	0.80	0.83	0.86	0.89	0.92	0.95	0.97	1.00	1.05	1.10	1.14	1.19	1.23
85	0.31	0.39	0.45	0.50	0.55	0.60	0.64	0.68	0.72	0.75	0.79	0.82	0.85	0.88	0.91	0.94	0.97	1.00	1.02	1.08	1.13	1.17	1.22	1.26
90	0.32	0.40	0.46	0.51	0.56	0.61	0.65	0.70	0.73	0.77	0.81	0.84	0.87	0.91	0.94	0.97	0.99	1.02	1.05	1.10	1.15	1.20	1.25	1.29
95	0.33	0.40	0.47	0.53	0.58	0.63	0.67	0.71	0.75	0.79	0.82	0.86	0.89	0.93	0.96	0.99	1.02	1.05	1.07	1.13	1.18	1.23	1.28	1.32
	12	**14**	**16**	**18**	**20**	**22**	**24**	**26**	**28**	**30**	**32**	**34**	**36**	**38**	**40**	**42**	**44**	**48**	**52**	**56**	**60**	**64**	**68**	**72**
100	0.59	0.64	0.68	0.73	0.77	0.81	0.84	0.88	0.91	0.95	0.98	1.01	1.04	1.07	1.10	1.13	1.15	1.21	1.26	1.30	1.35	1.40	1.44	1.48
105	0.60	0.65	0.70	0.74	0.78	0.82	0.86	0.90	0.93	0.97	1.00	1.03	1.06	1.09	1.12	1.15	1.18	1.23	1.28	1.33	1.38	1.43	1.47	1.52
110	0.61	0.67	0.71	0.76	0.80	0.84	0.88	0.92	0.95	0.99	1.03	1.05	1.08	1.11	1.14	1.17	1.20	1.25	1.31	1.36	1.41	1.45	1.50	1.55
115	0.63	0.68	0.73	0.77	0.81	0.86	0.89	0.93	0.97	1.00	1.04	1.07	1.10	1.13	1.16	1.19	1.22	1.28	1.33	1.38	1.43	1.48	1.53	1.58
120	0.64	0.69	0.74	0.79	0.83	0.87	0.91	0.95	0.99	1.02	1.06	1.09	1.12	1.15	1.19	1.22	1.24	1.30	1.36	1.41	1.46	1.51	1.56	1.60
125	0.65	0.70	0.75	0.80	0.84	0.89	0.93	0.97	1.00	1.04	1.07	1.11	1.14	1.17	1.21	1.24	1.27	1.32	1.38	1.43	1.49	1.54	1.58	1.63
130	0.66	0.71	0.77	0.81	0.86	0.90	0.94	0.98	1.02	1.06	1.09	1.13	1.16	1.19	1.23	1.26	1.29	1.35	1.40	1.46	1.51	1.56	1.61	1.66
135	0.67	0.73	0.78	0.83	0.87	0.92	0.96	1.00	1.04	1.07	1.11	1.15	1.18	1.21	1.25	1.28	1.31	1.37	1.43	1.48	1.53	1.59	1.64	1.69
140	0.68	0.74	0.79	0.84	0.89	0.93	0.97	1.01	1.05	1.09	1.13	1.16	1.20	1.23	1.26	1.30	1.33	1.39	1.45	1.50	1.56	1.61	1.66	1.71
145	0.69	0.75	0.80	0.85	0.90	0.94	0.99	1.03	1.07	1.11	1.14	1.18	1.22	1.25	1.28	1.32	1.35	1.41	1.47	1.53	1.58	1.64	1.69	1.74

	30	34	38	42	46	50	54	58	62	66	70	74	78	82	86	90	94	98	102	106	110	114	118	122
150	1.12	1.20	1.27	1.34	1.40	1.46	1.52	1.58	1.63	1.69	1.74	1.79	1.84	1.88	1.93	1.98	2.02	2.07	2.11	2.15	2.19	2.23	2.27	2.31
155	1.14	1.21	1.29	1.35	1.42	1.48	1.54	1.60	1.65	1.71	1.76	1.81	1.86	1.91	1.96	2.00	2.05	2.09	2.14	2.18	2.22	2.26	2.30	2.34
160	1.15	1.23	1.30	1.37	1.44	1.50	1.56	1.62	1.68	1.73	1.78	1.84	1.89	1.94	1.98	2.03	2.08	2.12	2.17	2.21	2.25	2.29	2.34	2.38
165	1.17	1.25	1.32	1.39	1.46	1.52	1.58	1.64	1.70	1.75	1.81	1.86	1.91	1.96	2.01	2.06	2.10	2.15	2.19	2.24	2.28	2.32	2.37	2.41
170	1.18	1.26	1.34	1.41	1.48	1.54	1.60	1.66	1.72	1.78	1.83	1.88	1.94	1.99	2.04	2.08	2.13	2.18	2.22	2.27	2.31	2.35	2.40	2.44
175	1.20	1.28	1.35	1.43	1.49	1.56	1.62	1.68	1.74	1.80	1.85	1.91	1.96	2.01	2.06	2.11	2.16	2.20	2.25	2.30	2.34	2.38	2.43	2.47
180	1.21	1.29	1.37	1.44	1.51	1.58	1.64	1.70	1.76	1.82	1.88	1.93	1.98	2.04	2.09	2.13	2.18	2.23	2.28	2.32	2.37	2.41	2.45	2.50
	40	**44**	**48**	**52**	**56**	**60**	**64**	**68**	**72**	**76**	**80**	**84**	**88**	**92**	**96**	**100**	**104**	**108**	**112**	**116**	**120**	**124**	**128**	**132**
185	1.42	1.49	1.56	1.63	1.69	1.75	1.81	1.87	1.93	1.98	2.03	2.08	2.13	2.18	2.23	2.28	2.33	2.37	2.42	2.46	2.50	2.55	2.59	2.63
190	1.44	1.51	1.58	1.65	1.71	1.77	1.83	1.89	1.95	2.00	2.06	2.11	2.16	2.21	2.26	2.31	2.35	2.40	2.44	2.49	2.53	2.58	2.62	2.66
195	1.45	1.53	1.60	1.67	1.73	1.79	1.85	1.91	1.97	2.02	2.08	2.13	2.18	2.23	2.28	2.33	2.38	2.43	2.47	2.52	2.56	2.60	2.65	2.69
200	1.47	1.54	1.62	1.68	1.75	1.81	1.87	1.93	1.99	2.05	2.10	2.15	2.21	2.26	2.31	2.36	2.40	2.45	2.50	2.54	2.59	2.63	2.68	2.72
205	1.49	1.56	1.63	1.70	1.77	1.83	1.89	1.95	2.01	2.07	2.12	2.18	2.23	2.28	2.33	2.38	2.43	2.48	2.52	2.57	2.62	2.66	2.70	2.75
210	1.50	1.58	1.65	1.72	1.79	1.85	1.91	1.97	2.03	2.09	2.14	2.20	2.25	2.30	2.36	2.41	2.45	2.50	2.55	2.60	2.64	2.69	2.73	2.77
215	1.52	1.59	1.67	1.74	1.80	1.87	1.93	1.99	2.05	2.11	2.17	2.22	2.27	2.33	2.38	2.43	2.48	2.53	2.58	2.62	2.67	2.71	2.76	2.80
220	1.53	1.61	1.68	1.75	1.82	1.89	1.95	2.01	2.07	2.13	2.19	2.24	2.30	2.35	2.40	2.45	2.50	2.55	2.60	2.65	2.69	2.74	2.79	2.83

Table B-3. Conversion Tables to Determine Body Surface Area (In Pounds and Inches)

Body surface area (m²) as a function of height (inches) and weight (pounds)*

Height (in)	Weight (lb)																							
	10	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**	**55**	**60**	**65**	**70**	**75**	**80**	**85**	**90**	**95**	**100**	**105**	**110**	**115**	**120**	**125**
20	0.27	0.33	0.38	0.43	0.47	0.51	0.55	0.58	0.62	0.65	0.68	0.70	0.73	0.76	0.78	0.81	0.83	0.86	0.88	0.90	0.92	0.94	0.97	0.99
22	0.28	0.34	0.40	0.45	0.49	0.53	0.57	0.61	0.64	0.67	0.70	0.73	0.76	0.79	0.82	0.84	0.87	0.89	0.92	0.94	0.96	0.98	1.01	1.03
24	0.29	0.30	0.41	0.47	0.51	0.55	0.59	0.63	0.66	0.70	0.73	0.76	0.79	0.82	0.85	0.87	0.90	0.93	0.95	0.97	1.00	1.02	1.04	1.07
26	0.30	0.37	0.43	0.48	0.53	0.57	0.61	0.65	0.69	0.72	0.76	0.79	0.82	0.85	0.88	0.90	0.93	0.96	0.98	1.01	1.03	1.06	1.08	1.10
28	0.31	0.38	0.44	0.50	0.55	0.59	0.63	0.67	0.71	0.75	0.78	0.81	0.84	0.87	0.90	0.93	0.96	0.99	1.01	1.04	1.06	1.09	1.11	1.14
30	0.32	0.39	0.46	0.51	0.56	0.61	0.65	0.69	0.73	0.77	0.80	0.84	0.87	0.90	0.93	0.96	0.99	1.02	1.04	1.07	1.10	1.12	1.15	1.17
32	0.33	0.40	0.47	0.53	0.58	0.62	0.67	0.71	0.75	0.79	0.82	0.86	0.89	0.92	0.96	0.99	1.02	1.04	1.07	1.10	1.13	1.15	1.18	1.20
34	0.34	0.41	0.48	0.54	0.59	0.64	0.69	0.73	0.77	0.81	0.85	0.88	0.92	0.95	0.98	1.01	1.04	1.07	1.10	1.13	1.16	1.18	1.21	1.23
36	0.34	0.42	0.49	0.55	0.61	0.66	0.70	0.75	0.79	0.83	0.87	0.90	0.94	0.97	1.00	1.04	1.07	1.10	1.13	1.16	1.18	1.21	1.24	1.26
38	0.35	0.43	0.50	0.57	0.62	0.67	0.72	0.76	0.81	0.85	0.89	0.92	0.96	0.99	1.03	1.06	1.09	1.12	1.15	1.18	1.21	1.24	1.27	1.29
	30	**35**	**40**	**45**	**50**	**55**	**60**	**65**	**70**	**75**	**80**	**85**	**90**	**95**	**100**	**105**	**110**	**115**	**120**	**125**	**130**	**135**	**140**	**145**
40	0.63	0.69	0.74	0.78	0.82	0.87	0.91	0.94	0.98	1.02	1.05	1.08	1.12	1.15	1.18	1.21	1.24	1.27	1.29	1.32	1.35	1.38	1.40	1.43
42	0.65	0.70	0.75	0.80	0.84	0.88	0.92	0.96	1.00	1.04	1.07	1.11	1.14	1.17	1.20	1.23	1.26	1.29	1.32	1.35	1.38	1.40	1.43	1.46
44	0.66	0.71	0.77	0.81	0.86	0.90	0.94	0.98	1.02	1.06	1.09	1.13	1.16	1.19	1.23	1.26	1.29	1.32	1.35	1.38	1.40	1.43	1.46	1.49
46	0.67	0.73	0.78	0.83	0.88	0.92	0.96	1.00	1.04	1.08	1.11	1.15	1.18	1.22	1.25	1.28	1.31	1.34	1.37	1.40	1.43	1.46	1.49	1.51
48	0.69	0.74	0.79	0.84	0.89	0.94	0.98	1.02	1.06	1.10	1.13	1.17	1.21	1.24	1.27	1.31	1.34	1.37	1.40	1.43	1.46	1.49	1.51	1.54
50	0.70	0.75	0.81	0.86	0.91	0.95	1.00	1.04	1.08	1.12	1.15	1.19	1.23	1.26	1.30	1.33	1.36	1.39	1.42	1.45	1.48	1.51	1.54	1.57
52	0.71	0.77	0.82	0.87	0.92	0.97	1.01	1.05	1.10	1.14	1.17	1.21	1.25	1.28	1.32	1.35	1.38	1.41	1.45	1.48	1.51	1.54	1.57	1.59
54	0.72	0.78	0.83	0.89	0.94	0.98	1.03	1.07	1.11	1.15	1.19	1.23	1.27	1.30	1.34	1.37	1.41	1.44	1.47	1.50	1.53	1.56	1.59	1.62
56	0.73	0.79	0.85	0.90	0.95	1.00	1.04	1.09	1.13	1.17	1.21	1.25	1.29	1.32	1.36	1.39	1.43	1.46	1.49	1.52	1.55	1.59	1.62	1.64
58	0.74	0.80	0.86	0.91	0.97	1.01	1.06	1.10	1.15	1.19	1.23	1.27	1.31	1.34	1.38	1.41	1.45	1.48	1.51	1.55	1.58	1.61	1.64	1.67

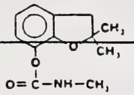
	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280
60	0.98	1.08	1.16	1.25	1.32	1.40	1.47	1.54	1.60	1.66	1.72	1.78	1.84	1.89	1.95	2.00	2.05	2.10	2.15	2.19	2.24	2.29	2.33	2.38
62	0.99	1.09	1.18	1.26	1.34	1.42	1.49	1.56	1.62	1.69	1.75	1.81	1.86	1.92	1.97	2.03	2.08	2.13	2.18	2.23	2.27	2.32	2.36	2.41
64	1.01	1.11	1.20	1.28	1.36	1.44	1.51	1.58	1.65	1.71	1.77	1.83	1.89	1.94	2.00	2.05	2.11	2.16	2.21	2.26	2.30	2.35	2.40	2.44
66	1.02	1.12	1.21	1.30	1.38	1.46	1.53	1.60	1.67	1.73	1.79	1.85	1.91	1.97	2.03	2.08	2.13	2.18	2.24	2.28	2.33	2.38	2.43	2.47
68	1.03	1.13	1.23	1.31	1.40	1.47	1.55	1.62	1.69	1.75	1.82	1.88	1.94	2.00	2.05	2.11	2.16	2.21	2.26	2.31	2.36	2.41	2.46	2.50
70	1.04	1.15	1.24	1.33	1.41	1.49	1.57	1.64	1.71	1.78	1.84	1.90	1.96	2.02	2.08	2.13	2.19	2.24	2.29	2.34	2.39	2.44	2.49	2.54
72	1.06	1.16	1.26	1.35	1.43	1.51	1.59	1.66	1.73	1.80	1.86	1.92	1.98	2.04	2.10	2.16	2.21	2.27	2.32	2.37	2.42	2.47	2.52	2.57
	90	**100**	**110**	**120**	**130**	**140**	**150**	**160**	**170**	**180**	**190**	**200**	**210**	**220**	**230**	**240**	**250**	**260**	**270**	**280**	**290**	**300**	**310**	**320**
74	1.45	1.53	1.61	1.68	1.75	1.82	1.88	1.96	2.01	2.07	2.13	2.18	2.24	2.29	2.35	2.40	2.45	2.50	2.55	2.60	2.64	2.69	2.74	2.78
76	1.46	1.55	1.62	1.70	1.77	1.84	1.90	1.97	2.03	2.09	2.15	2.21	2.26	2.32	2.37	2.43	2.48	2.53	2.58	2.63	2.67	2.72	2.77	2.81
78	1.48	1.56	1.64	1.72	1.79	1.86	1.93	1.99	2.05	2.11	2.17	2.23	2.29	2.34	2.40	2.45	2.50	2.55	2.61	2.65	2.70	2.75	2.80	2.84
80	1.50	1.58	1.66	1.73	1.81	1.88	1.95	2.01	2.08	2.14	2.20	2.26	2.31	2.37	2.42	2.48	2.53	2.58	2.63	2.68	2.73	2.78	2.83	2.87
82	1.51	1.60	1.68	1.75	1.83	1.90	1.97	2.03	2.10	2.16	2.22	2.28	2.34	2.39	2.45	2.50	2.56	2.61	2.66	2.71	2.76	2.81	2.86	2.90
84	1.53	1.61	1.69	1.77	1.85	1.92	1.99	2.05	2.12	2.18	2.24	2.30	2.36	2.42	2.48	2.53	2.58	2.64	2.69	2.74	2.79	2.84	2.89	29.3
86	1.54	1.63	1.71	1.79	1.86	1.94	2.01	2.07	2.14	2.20	2.27	2.33	2.39	2.44	2.50	2.56	2.61	2.66	2.71	2.77	2.82	2.87	2.91	2.96

MATERIAL SAFETY DATA SHEET

DATA SHEET NO.	7019
DIVISION	Chemagro
ISSUE DATE	10/1/82
ISSUED BY	CDS
SUPERSEDES ISSUE OF	None

MANUFACTURING DIV. ADDRESS

CHEMAGRO LTD.
1355 Aerowood Drive
Mississauga, Ontario, Canada L4W 1C2

CHEMTREC CHEMICAL TRANSPORTATION EMERGENCY TELEPHONE NO: 800-424-9300; DISTRICT OF COLUMBIA: 202-483-7616	MOBAY NON-TRANSPORTATION EMERGENCY NO.: (416)625-5280 CANUTEC Emergency No.: (613)996-6666
PRODUCT NAME FURADAN 480F F= Flowable	PRODUCT CODE NUMBER Formula No. 062307
CHEMICAL FAMILY Carbamate Pesticide	CHEMICAL NAME & SYNONYMS 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate
CHEMICAL FORMULA 	TRADE NAME & SYNONYMS Carbofuran

HAZARDOUS INGREDIENTS

HAZARDOUS MIXTURES OF OTHER LIQUIDS, SOLIDS OR GASES	%	CURRENT TLV
FURADAN	41	0.1 mg/m^3

PHYSICAL DATA

APPEARANCE (SOLID, LIQUID, GAS) Liquid	MOLECULAR WEIGHT 221.25 (A.I.)	MELT POINT 0 °C	SPECIFIC GRAVITY 1.10 kg/L
VAPOR DENSITY (AIR=1) NE	COLOR Tan to brown	BULK DENSITY NA	BOILING POINT NE
VAPOR PRESSURE NE	SOLUBILITY (WATER) Insoluble (A.I.)	ODOR Slight phenol	% VOLATILE BY VOLUME NE

FIRE & EXPLOSION DATA

FLASH POINT °F (METHOD USED) NA	FLAMMABLE LIMIT Lel NA Uel NA	EXTINGUISHING MEDIA Water Spray

SPECIAL FIRE FIGHTING PROCEDURES, UNUSUAL FIRE OR EXPLOSION HAZARDS

Wear self-contained breathing equipment. Stay out of smoke. Cool exposed containers with water spray.

None

TOXICITY DATA

LD50, ORAL (INGESTION) Rat 19 mg/kg	LD50, DERMAL (SKIN CONTACT) Rabbit 6,800 mg/kg	INHALATION (LC50) NE
FISH, LC50 (LETHAL CONCENTRATION) NE	TLV (UNITS) (THRESHOLD LIMIT VALUE) (FURADAN) 0.1 mg/m^3	SKIN IRRITATION NE
EFFECTS TO EYE NE	EFFECTS TO LUNG NE	OTHER NE

EMERGENCY AND FIRST AID PROCEDURES, EFFECTS OF OVER EXPOSURE

If swallowed, induce vomiting. Remove contaminated clothing and flush eyes and skin with water. Shower thoroughly, washing skin and hair with soap and water. Symptoms of overexposure are headache, dizziness, ataxia, constricted pupils, nausea, vomiting, sweating, respiratory depression, blood cholinesterase depression. Consult physician.

REACTIVITY DATA

Formula No. 062307

STABILITY Stable	CONDITIONS TO AVOID Sustained temperatures above 38°C
POLYMERIZATION None	CONDITIONS TO AVOID

INCOMPATABILITY (MATERIALS TO AVOID)
Strong alkali

HAZARDOUS DECOMPOSITION PRODUCTS
CO, Methyl Amine, CO_2, CH_3CNO

SPILL OR LEAK PROCEDURE

TEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED

Avoid skin contact and breathing vapors. Absorb spilled material. Apply lime and bleach to the contaminated area and sweep up. Wash area with detergent and water.

WASTE DISPOSAL METHOD

Bury in hazardous materials landfill approved by the Government Ministry responsible or burn in an incinerator approved for pesticide destruction.

SPECIAL PROTECTION DATA

RESPIRATOR TYPE

NIOSH-approved chemical cartridge or full-face respirator with pesticide cartridges

EYE PROTECTION Splash-proof goggles	GLOVES Latex or Neoprene

OTHER PROTECTIVE EQUIPMENT

Rubber boots and apron. Launder clothing daily after use

SPECIAL PRECAUTIONS & STORAGE DATA

STORAGE TEMPERATURE (OPTIMUM) Min. 0°C Max. 49°C	AVERAGE SHELF LIFE

SPECIAL SENSITIVITY (HEAT, LIGHT, MOISTURE)
Heat

RECAUTIONS TO BE TAKEN IN HANDLING AND STORING

DO NOT store near any material intended for use or consumption by humans or animals.

SHIPPING DATA

.O.T. SHIPPING NAME Insecticides, Liquid		TECHNICAL SHIPPING NAME Carbofuran	
O.T. HAZARD CLASSIFICATION Poison B	UN/NA NO. 2757	R.Q. 10 lbs.	
O.T. LABELS REQUIRED Poison	LABEL	T.S.C.A. STATUS NA	
EASON FOR ISSUE: Original Issue	FRT. CLASS BULK: FRT. CLASS PKG:		

ITIATED BY TITLE: Larry R. Thompson, Industrial Hygienist	APPROVED BY: TITLE: Wm. J. Brinkman, Industrial Hygiene Manager
ATE INITIATED Oct. 1, 1982 Larry Thompson	DATE APPROVED: October 1, 1982 W.J. Brinkman

N E NOT ESTABLISHED N A = NOT APPLICABLE A I = ACTIVE INGREDIENT

from: Guide to Crop Protection in Alta. Part 1 - Chemica
Alta Agriculture

FURADAN (carbofuran)

1. FORMULATIONS: Granular – 5G (5% carbofuran)
   – 10G and CR-10 (10% carbofuran)
   Flowable – (480 g carbofuran/L)

2. MARKETING CATEGORY: Commercial

3. REGISTERED MIXES: Flowable formulation may be tank-mixed with phenoxy ester or amine herbicides and should be used only on crops listed on both lables. Compatible with most fungicides. Do not mix with Bordeaux or hydrated lime.

4. HOW IT WORKS: Carbofuran is a broad-spectrum, systemic, carbamate insecticide, acaricide and nematicide which works by contact.

5. NUMBER OF APPLICATIONS:

   Granular – one per season.
   Flowable: Oilseeds – for flea beetle control, one application at 110 mL/ac **(275 mL/ha)** or two applications totalling not more than 110 mL/ac **(275 mL/ha).**
   Grains and forages – two applications at 110 mL/ac **(275 mL/ha)** or, for alfalfa weevil, one at 225 mL/ac **(550 mL/ha)**.
   Corn – four applications for corn borer.
   Potatoes – repeat as necessary.

6a. HOW TO APPLY: Granular

Rate:

Crop	**Insect**	**Formulation**	**kg/ac**	**(kg/ha)**
Canola, mustard	Flea beetles	5G	1.8-2.2	**(4.5-5.5)**
		or CR-10	1.1	**(2.8)**
Potato	Colorado potato beetle, potato flea beetle, potato leafhoppers	10G	13.2 Using 90 cm row spacing or 300 g/100 m of row.	**(32.5)**
Sugar beet	Sugar beets root maggot	10G	3.4	**(8.5)**

Note: – Use the high rate if a severe infestation is anticipated.

Incorporation: Canola, mustard – for seed drill application only; not valid for application with discer seeders. Do not harrow after seeding. Mix granules and seed thoroughly. Check for accurate calibration.
Potatoes – apply as a 10 cm wide band into seed furrow or drill into the soil 10 cm on each side of row and 5 cm below seed.
Sugar beets – apply directly into seed furrow at same depth as seed or slightly above seed. Do not mix seed, fertilizer and insecticide in same hopper.

Protective Equipment: Wear protective clothing and, if exposure to dust cannot be avoided, a pesticide respirator and goggles.

6b. HOW TO APPLY: Flowable

With: Ground or aircraft equipment.

Rate:

Crop	**Insect**	**mL/ac**	**(mL/ha)**
Alfalfa (7)	Alfalfa weevil	225	(550)
Alfalfa (1), barley (21), canola(60), corn – sweet (7), field (3) flax (21), mustard (21), oats (21), pasture (1), sweet clover (28), wheat (21)	Grasshoppers	110	(275)
Canola (60), mustard (21)	Flea beetles	60-110	(150-275)

*******LABEL RATES ARE GIVEN ONLY IN QUANTITY OF PRODUCT PER HECTARE******

	Red turnip beetle	110	(275)
Corn (field, silage, sweet) (7)	European corn borer	445	(1100)
Potato(7)	Any of: aphids, potato flea beetle, potato leafhopper or tarnished plant bug alone or in combination with Colorado potato beetle.	445	(1100)
Potato(7)	Colorado potato beetle	225	(550)

Notes: 1. Pre-harvest or pre-grazing interval (days) given in brackets after crop.
2. Canola – use the high rate for severe infestations.
3. Potatoes – use 690 mL/ac (1.75 L/ha) if large nubmers of aphids are present.

Water Volume: Ground application – not less than 40 L/ac (100 L/ha), use sufficient water for thorough coverage. Aerial application – not less than 20 L/ac (50 L/ha). Potatoes – use 325-405 L/ac (800-1000 L/ha) at a minimum pressure of 875 kPa.

Protective Equipment: Wear protective clothing, a pesticide respirator and goggles.

7. SPRAYING TIPS: Check the label for calibration of various types of granular applicators. If seed decay, seedling blight or damping-off diseases are a problem, treat seed with a recommended fungicide. Canola and mustard may also require a foliar treatment after seeding with granules. Check fields shortly after emergence. Alfalfa – apply when 25% of tips show feeding damage by alfalfa weevil. In general, apply foliar sprays when insects or feeding damage first appears. Boom sprayers – equip with hydraulic or mechanical agitation and 0.3 mm screens; remove any felt filters.

8. GRAZING RESTRICTIONS: Sugar beet tops and pulp may be fed to livestock without causing residues in milk or meat. Note grazing wait periods in section 6b.

9. TOXICITY: Acute oral LD_{50} = 11 mg/kg (rat)

10. SAFETY:

Symptoms of poisoning: Typical cases involving blurred vision, nausea, excessive perspiration and a sense of weakness have been reported in formulators and applicators. Additional symptoms may include headache, light-headedness, constriction of pupils, cramps, salivation and vomiting.

Toxicological information (for the physician): Carbofuran is a reversibie cholinesterase inhibitor. Give atropine 2 mg intravenously. If in eyes, instill one drop of Homatropine. Do not use oximes such as 2-PAM.

First aid: IF SWALLOWED – vomiting should be induced. **Do not induce vomiting in a convulsive or unconscious person.** Administer milk or water freely and induce vomiting by giving one dose (15 ml) of syrup of ipecac. If vomiting does not occur within 10 to 20 minutes, administer a second dose. If syrup of ipecac is not available, induce vomiting by sticking finger down throat. Repeat until vomit fluid is clear. Patient should be lying face down or on side with the head below foot level. Professional medical assistance should be secured immediately.

IF INHALED – have victim lie down and remain quiet.

IF ON SKIN – remove contaminated clothing and immediately wash skin with soap and water.

IF IN EYES – flush eyes with plenty of water for at least 15 minutes. Call a doctor immediately.

11. PRECAUTIONS:

KEEP OUT OF REACH OF CHILDREN.

DANGER – Do not breathe spray mist or dust. Wear goggles at all times. Wear a respirator when breathing of spray mist or dust is unavoidable.

Wear coveralls at all times. Never handle product with bare hands – use rubber or neoprene gloves. Do not use leather or cloth gloves.

*******LABEL RATES ARE GIVEN ONLY IN QUANTITY OF PRODUCT PER HECTARE******

Wash hands, face, neck and arms thoroughly after handling product and before eating or smoking. Take a complete bath at end of day. Change clothing each day after using product. Wash clothes in detergent, bleach and hot water.

Very poisonous to bees – do not apply when crops are in bloom or allow spray to drift toward beehives. Applications by both ground and air are permitted, provided there is no hazard of drift or application to areas occupied by people or livestock. Remove cattle before spraying. Do not contaminate feed or food. Keep out of areas inhabited by fish, birds and wildlife – this insecticide is highly toxic to such animals.

12. STORAGE: Store away from food and feed. Do not store in or around the home. Store in a safe, cool place. Protect flowable from freezing.

13. DISPOSAL: Puncture and bury empty containers at least 50 cm deep away from water supply in a non-crop, non-graze area. Do not reuse empty container.

14. WHERE AVAILABLE: Alberta Wheat Pool, Oliver Agricultural Supplies, Cargill Grain, Pfizer Chemical Dealers, Pioneer Grain.

Additional information available from:

Chemagro Ltd.
1355 Aerowood Drive
Mississauga, Ontario
L4W 1C2
Phone: (416) 625-5280

Chemagro Ltd.
#203, 610 – 7th Ave. S.E.
Calgary, Alberta
T2H 2J6
Phone: (403) 259-2863

*******LABEL RATES ARE GIVEN ONLY IN QUANTITY OF PRODUCT PER HECTARE******

EPA CHEMICAL PROFILE

INTERIM
Date: October 31, 1985
Revision: ____________

CHEMICAL IDENTITY -- CARBOFURAN

CAS Registry Number: 1563-66-2

Synonyms: Carbamic Acid, Methyl-, 2,3-Dihydro-2,2-Dimethyl-7-Benzofuranyl Ester; 2,2-Dimethyl-2,2-Dihydrobenzofuranyl-7 N-Methylcarbamate; 2,3-Dihydro-2,2-Dimethyl-7-Benzofuranol-N-Methylcarbamate; 2,3-Dihydro-2,2-Dimethylbenzofuranyl Methylcarbamate; 2,3-Dihydro-2,2-Dimethylbenzofuranyl-7-N-Methylcarbamate; 7-Benzofuranol, 2,3-Dihydro-2,2-Dimethyl-, Methylcarbamate; Bay 70143; Chinufur; Curaterr; D 1221; ENT 27,164; FMC 10242; Furadan; Furadan 3G; Furodan; NIA 10242; Niagara 10242; Niagara Nia-10242; OMS 864; Yaltox; 7-Benzofuranol, 2,3-Dihydro-2,2-Dimethyl-, Methylcarbamate

Chemical Formula: $C_{12}H_{15}NO_3$

Molecular Weight: 221.28

SECTION I -- HAZARDOUS INGREDIENTS/IDENTITY INFORMATION

OSHA PEL: Not Found

ACGIH TLV: TWA 0.1 mg/m^3 (*ACGIH 1983)

IDLH: Not Found

Other Limits Recommended: Toxicity information: LC_{50} inhalation (guinea pig) 0.043 mg/liter for 4 hours (*NIOSH/RTECS 1985)

SECTION II -- PHYSICAL/CHEMICAL CHARACTERISTICS

Boiling Point: Not Found

Specific Gravity (H_2O=1): 1.18 at 20°C (*Farm Chemicals Handbook 1984)

Vapor Pressure (mmHg): 2×10^{-5} at 33°C (*Farm Chemicals Handbook 1984)

Melting Point: 302-307°F, 150-153°C (*Merck 1983)

Vapor Density (AIR=1): Not Found

CARBOFURAN

SECTION II -- PHYSICAL/CHEMICAL CHARACTERISTICS (Continued)

Evaporation Rate (Butyl acetate=1): Not Found

Solubility in Water: 700 ppm at 25°C (*Merck 1976)

Appearance and Odor: White crystalline solid (*Merck 1976); odorless (*Farm Chemicals Handbook 1984)

SECTION III -- FIRE AND EXPLOSION HAZARD DATA

Flash Point (Method Used): Not Found
Flammable Limits:
  LEL: Not Found
  UEL: Not Found

Extinguishing Methods: Use dry chemical, carbon dioxide, water spray, or foam. Dike fire control water for later disposal, do not scatter the material (*DOT 1984).

Special Fire Fighting Procedures: Stay at maximum distance (*DOT 1984).

Unusual Fire and Explosion Hazards: May release nitrogen oxides (Sax 1984, p. 1152). Containers may explode in heat of fire (*DOT 1984).

SECTION IV -- REACTIVITY DATA

Stability: **Unstable:**
  Stable: Yes (Under neutral or acid conditions (*Spencer 1982)

Conditions to Avoid: Not Found

Incompatibility (Materials to Avoid): Alkalies (*Merck 1976)

Hazardous Decomposition or Byproducts: Nitrogen oxides (Sax 1984, p. 1152)

Hazardous Polymerization: **May Occur:** Not Found
  May Not Occur: Not Found

Conditions to Avoid: Not Found

CARBOFURAN

SECTION V -- HEALTH HAZARD DATA

Routes of Entry: **Inhalation:** Yes (*DOT 1984)
Skin: Yes (*DOT 1984)
Ingestion: Yes (*DOT 1984)

Health Hazards (Acute, Delayed, and Chronic): This material is extremely poisonous. May be fatal if swallowed, inhaled, or absorbed through skin. Contact may burn skin or eyes (*DOT 1984). Probable lethal oral dose to humans 5 to 50 mg/kg or 7 drops to 1 teaspoon for 150 lb. person (*Gosselin 1976).

Signs and Symptoms of Exposure: Symptoms include headache, giddiness, blurred vision, weakness; nausea, cramps, diarrhea, chest discomfort, sweating, contraction of pupils, tearing; salivation, blue lips, lungs and abdomen fill with fluid, convulsions, coma, loss of reflexes and sphincter control (*Gosselin 1976).

Medical Conditions Generally Aggravated by Exposure: Not Found

Emergency and First Aid Procedures: Move victim to fresh air; call emergency medical care. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. In case of contact with material, immediately flush skin or eyes with running water for at least 15 minutes. Speed in removing material from skin is of extreme importance. Remove and isolate contaminated clothing and shoes at the site. Keep victim quiet and maintain normal body temperature. Effects may be delayed; keep victim under observation (*DOT 1984).

SECTION VI -- USE

This material is used as an insecticide on corn, alfalfa, tobacco and other field crops (*SRI).

SECTION VII -- PRECAUTIONS FOR SAFE HANDLING AND USE

(Steps to be Taken in Case Material is Released or Spilled)

In case of releases or spills, stay upwind; keep out of low areas. Ventilate closed spaces before entering them. Wear positive pressure breathing apparatus and special protective clothing (*DOT 1984).

FORM 58M38

WORKERS' COMPENSATION BOARD OF BRITISH COLUMBIA
6951 Westminster Highway, Richmond, B.C. V7C 1C6, Telephone 273-2266, Telex 04-357722

LABORATORY SERVICES

ANALYTICAL METHODS

No. 2200

PAGE 1 OF 12

DATE 19 OCT 84

REPLACES ISSUE DATED 11 MAY 83

Subject: CHOLINESTERASE IN WHOLE BLOOD AND PLASMA

1.0 PRINCIPLE

1.1 Acetylcholinesterase and pseudocholinesterase are enzymes whose activity is supressed in response to exposure to organophosphate and carbamate pesticides. Supression of pseudocholinesterase (CHE) activity takes place 24 - 72 hours after exposure and acetylcholinesterase (ACHE) activity, approximately 36 hours after exposure. The enzyme activity then returns back to normal over a one-week period depending on severity of exposure. The measurement of the acetylcholinesterase activity in red blood cells (RBC) and the pseudocholinesterase activity in blood plasma can be used to assess organophosphate or carbamate exposure. A haemoglobin determination is also performed so that the acetylcholinesterase activity can be expressed in activity units per gram of haemoglobin. For the cholinesterase results to be meaningful the time between exposure and sample collection must be known.

1.2 The activity of the enzymes is determined kinetically by measuring the rate of enzyme hydrolysis of a substrate (acetylthiocholine iodide or benzoylcholine iodide) spectrophotometrically as a function of time. The slope for the graph of absorbance against time is equal to the reaction rate which is used to calculate the respective enzyme activities.

1.3 For the cholinesterase activity measurements to be meaningful it is necessary to establish the normal cholinesterase activity of each individual worker prior to exposure. The workers are then sampled through the year as suspected exposures take place.

2.0 RANGE

2.1 Haemoglobin: 6 - 18 g/100 mL of whole blood.

2.2 Acetylcholinesterase: 100 - 500 umole L^{-1} min^{-1}(g Haemoglobin $dL^{-1})^{-1}$ at 25°C.

2.3 Pseudocholinesterase: 100 - 1300 umole L^{-1} min^{-1} at 25°C.

3.0 PRECISION AND ACCURACY

3.1 To be determined.

4.0 INTERFERENCES

4.1 The enzyme activity can be depressed by biological disorders, such as:

a) CHE: anaemia, shock, liver disorders, T.B., congenital defect.

b) ACHE: cardiac failure.

5.0 ADVANTAGES AND DISADVANTAGES

5.1 The method is sensitive, fast, and relatively simple to perform.

5.2 Temperature and pH are critical. Temperature must be stabilized to 25 ± 0.1°C for both enzyme analyses. For ACHE the pH of the reaction medium is 8.0 ± 0.1 and for CHE pH is 7.4 ± 0.1.

5.3 A depressed enzyme activity is not specific for pesticide exposure. A clinical examination and/or other clinical analyses will be necessary to confirm this.

6.0 APPARATUS

6.1 Sample Collection

6.1.1 Vacutainer tubes (Sodium Heparin - green stopper) - 10 mL capacity (Becton Dickinson Cat. No. 6480 or equivalent).

6.1.2 Vacutainer holder (Becton Dickinson Cat. No. 4893 or equivalent).

6.1.3 Vacutainer multi-sample needles (guage 21) (Becton Dickinson Cat. No. 7212 or equivalent).

6.1.4 Miscellaneous items including cotton balls or guaze pads, adhesive bandages, alcohol wipes (Becton-Dickinson Cat. No. 102-63000 or equivalent).

6.2 Sample Analysis

6.2.1 Centrifuge (Clay Adams or equivalent).

6.2.2 Vortex Mixer (Fisher Cat. No. 12-812 or equivalent).

6.2.3 Pasteur Pipettes.

6.2.4 Water Purification System (Millipore Milli-Q System or equivalent).

6.2.5 A UV-VIS Spectrophotometer (Perkin Elmer Lambda 3B or equivalent) equipped with temperature controlled flow-through sample cell (Perkin Elmer Supersipper C-005-0602 or equivalent).

6.2.6 Chart Recorder (Perkin Elmer Model R100A or equivalent).

6.2.7 Balance capable of weighing to within ± 0.01 mg (Mettler or equivalent).

6.2.8 pH meter (Corning Digital 110 or equivalent).

6.2.9 Water Bath (15-20 gal. capacity) capable of maintaining a temperature of 25 ± 0.1°C.

6.2.10 Shaker (Labquake™ or equivalent).

6.2.11 Parafilm™.

6.2.12 Cells: one thermostatted micro-flow cell (Perkin Elmer C-005-0155) and one - 1 cm quartz cell.

6.2.13 Disposable 20 uL Micro-pipettes (Fisher Cat. No. 21-164-2D or equivalent).

6.2.14 Multivolume Micropipette - 10-25 uL (Fisher Cat. No. 21-380-6D or equivalent). Pipet Tips (Fisher Cat. No. 21-381-10A or equivalent).

6.2.15 Test Tubes - 12 mL disposable (Sarstedt Cat. No. 57/527 or equivalent)
and caps for tubes (Sarstedt Cat. No. 65-793 or equivalent).

7.0 REAGENTS

All reagents should be reagent grade or better. Water - purified (Milli-Q or equivalent).

7.1 Haemoglobin

7.1.1 Drabkin's Reagent Prepare by dissolving in 1 litre of water:

(i) 0.20 g Potassium Ferricyanide ($K_3Fe[CN]_6$)
(ii) 0.05 g Potassium Cyanide (KCN)
(iii) 1.0 g Sodium Bicarbonate ($NaHCO_3$)
Store in a dark brown container at 4°C

7.1.2 Haemoglobin Standard Set (Fisher Cat. No. 251-18 or equivalent) Reconstituted standard can be kept approximately 12 months if stored at 4°C.

7.2 Acetylcholinesterase

7.2.1 Sodium Dihydrogen Phosphate Monohydrate 0.1M - Dissolve 13.80 g of $NaH_2PO_4 \cdot H_2O$ in 1000 mL of water.

7.2.2 Disodium Hydrogen Phosphate Dodecahydrate 0.1M - Dissolve 35.8 g of $Na_2HPO_4 \cdot 12H_2O$ in 1000 mL of water.

7.2.3 Phosphate Buffer - 0.1M pH 7.0 - Mix 40.0 mL of 7.2.1 and 60.0 mL of 7.2.2. Check the pH. If the pH is not equal to 7.0, adjust to the desired pH with stock solution of either 7.2.1 or 7.2.2.

7.2.4 Phosphate Buffer 0.1M pH 8.0 - Mix 30 mL of 7.2.1 and 500 mL of 7.2.2. Check the pH. If the pH is not equal to 8.0, adjust to the desired pH with stock solution of either 7.2.1 or 7.2.2.

7.2.5 Acetylthiocholine Iodide Substrate 75mM (Sigma Cat. No. A5751 or equivalent) Prepare fresh as required. Weigh 217.0 mg of the substrate and dissolve in 10 mL of water. Mix gently by inversion several times.

7.2.6 Sodium Bicarbonate ($NaHCO_3$)

7.2.7 5,5'-Dithiobis-(2-nitrobenzoic acid) - (DTNB) (Sigma Cat. No. D8130 or equivalent). Dissolve 39.6 mg of DTNB in 10 mL of 7.2.3. Add 15 mg of $NaHCO_3$. Mix well. This solution is stable for 30 days if stored at 4°C.

7.2.8 Quinidine Sulphate 0.1% (Sigma Cat. No. Q0875 or equivalent) Dissolve 12.1 mg of quinidine sulphate in 10 mL of water. Prepare fresh as required or store in a dark bottle at 4°C.

7.3 Pseudocholinesterase

7.3.1 Disodium Hydrogen Phosphate 0.067M - Dissolve 9.47 g of anhydrous Na_2HPO_4 in 1000 mL water.

7.3.2 Potassium Dihydrogen Phosphate 0.067M - Dissolve 9.08 g of KH_2PO_4 in 1000 mL water.

7.3.3 Sorenson's Phosphate Buffer 0.067M pH 7.4 - Mix 800 mL of 7.3.1 and 200 mL of 7.3.2. Check the pH. If the pH is not equal to 7.4, adjust to the desired pH with stock solution of either 7.3.1 or 7.3.2.

7.3.4 Benzoylcholine Iodide Stock Solution 0.0025 M (Sigma Cat. No. B6257 or equivalent). Weigh 42 mg of the substrate into a 50 mL volumetric flask and adjust to volume with Sorenson's phosphate buffer pH 7.4 (7.3.3). Prepare fresh for use.

7.3.5 Benzoylcholine Iodide Substrate Working Solution 0.00005M - Pipette 10 mL of 7.3.4 and dilute to 500 mL with Sorenson's phosphate buffer pH 7.4 (7.3.3). Prepare fresh for use.

7.3.6 Pseudocholinesterase Control Sera Level I and Level II (Fisher Cat. No. 2907-63 or equivalent). Reconstitute as per manufacturer's directions. Divide into 0.25 mL aliquots and freeze at -20°C.

8.0 PROCEDURE

8.1 Sample Collection

8.1.1 Collect 10 mL of blood in a vacutainer tube.

8.1.2. The tube should be labelled with the worker's name and date of sample collection. Date of last pesticide exposure should be recorded on the analytical request form.

8.1.3 The sample should be refrigerated after collection and delivered to the laboratory within 72 hours of collection.

8.1.4 Upon arrival in the Laboratory, the sample should be mixed well by shaking gently and split into two vacutainers. One tube should be centrifuged at 3000 r.p.m. for ten minutes and the plasma (top clear yellow fluid) separated from the red blood cells and placed in a vial, using a disposable pasteur pipet. The whole blood (for ACHE) and plasma (for CHE) should be refrigerated. If analysis is not immediate, the plasma should be frozen.

8.2 Sample Analysis - Haemoglobin

Note: Haemoglobin analysis should be performed the same day as acetylcholinesterase.

8.2.1 a) Set the spectrophotometer at the following conditions:

Reference Cell	1 cm quartz
Sample Cell	quartz micro-flow cell
Mode	absorbance
Wavelength	540 nm

b) Set the sampler at the following conditions:

Temperature	off
Sample Volume	2 mL

8.2.2 Mix the vacutainer of whole blood on the shaker until the sample is homogeneous.

8.2.3 Pipette 5.0 mL of Drabkin's reagent (7.1.1) into a 12 mL disposable test tube.

8.2.4 Dispense 20 uL of whole blood using a disposable micro-pipette. Rinse the pipette repeatedly in the Drabkin's reagent (8.2.3).

8.2.5 Mix thoroughly using the vortex mixer and allow five minutes for color development.

8.2.6 Read the absorbance against a reagent blank (Drabkin's reagent 7.1.1) and determine the haemoglobin level per 100 mL whole blood from the calibration graph prepared in Section 9.0 below.

8.3 Sample Analysis - Acetylcholinesterase

Note: Before performing the analysis, the chart recorder and spectrophotometer must be checked to ensure that an absorbance change on the spectrophotometer corresponds to the absorbance change measured on the chart recorder paper.

Perform the analysis one sample at a time in duplicate.

8.3.1 a) Set the spectrophotometer at the following conditions:

Reference Cell	1 cm quartz
Sample Cell	micro-flow cell
Wavelength	412 nm
Mode	absorbance
Ordinate limits	as required for reaction to be on scale on chart recorder

b) Set the sampler at the following conditions:

Temperature	$25 \pm 0.1^{\circ}C$
Sample Volume	2 mL

c) Set the recorder at the following conditions:

Chart Speed	10 mm/min
Range	1000 mV

8.3.2 Zero the spectrophotometer and recorder with phosphate buffer pH 8.0 in the reference and sample cells.

8.3.3 Mix the vacutainer of whole blood on the shaker until the sample is homogeneous. Into a 12 mL test tube con- taining 6.0 mL of phosphate buffer pH 8.0 (7.2.4) dispense 10 uL of whole blood. Cap and mix thoroughly.

8.3.4 Sequentially add the following to another 12 mL test tube:

- i 3.0 mL of diluted sample (8.3.3)
- ii 10 uL of 0.1% quinidine sulphate (7.2.8)
- iii 25 uL of DTNB (7.2.7)

Equilibrate the tube for five minutes in a $25 \pm 0.1^{\circ}C$ waterbath.

8.3.5 Add 20 uL of 75 mM acetylthiocholine substrate (7.2.5) to the tube. Cap and mix thoroughly.

8.3.6 Transfer the sample to the spectrophotometer sample cell using the sample sipper and measure the change in absorbance over the linear portion of the graph (See Appendix I).

8.3.7 Calculate acetylcholinesterase activity according to Section 10.2 below.

8.4 Sample Analysis - Pseudocholinesterase

Note: Before performing the analysis, the chart recorder and spectrophotometer must be checked to ensure that an absorbance change on the spectrophotometer corresponds to the absorbance change measured on the chart recorder paper.

Perform the analysis one sample at a time in duplicate.

8.4.1 a) Set the spectrophotometer at the following conditions:

Reference Cell	1 cm Quartz
Sample Cell	Micro-flow cell
Wavelength	240 nm
Mode	absorbance
Ordinate Limits	as required for reaction to be on scale on chart recorder

b) Set the sampler at the following conditions:

Temperature	25 ± 0.1°C
Sample Volume	2 mL

c) Set the recorder at the following conditions

Chart Speed	10 mm/min
Range	1000 mV

8.4.2 If the plasma sample is frozen, thaw at room temperature and mix by inversion before analysing.

8.4.3 Zero the spectrophotometer and recorder with Sorenson's phosphate buffer (7.3.3) in the reference and sample cells.

8.4.4 Pipette 4.0 mL of Benzoylcholine substrate (7.3.5) into a 12 ml test tube and equilibrate to 25 ± 0.1°C in a water batch (five minutes).

8.4.5 Add 20 uL of plasma, cap the tube, mix well by vortexing and transfer to the spectrophotometer sample cell using the sipper.

8.4.6 Measure the change in absorbance over the linear portion of the graph (See Appendix I).

8.4.7 Calculate pseudocholinesterase activity according to Section 10.3 below.

9.0 CALIBRATION AND STANDARDS

9.1 Haemoglobin Standard

9.1.1 Set the spectrophotometer at the analysis conditions outlined in Section 8.2.1.

9.1.2 Zero the spectrophotometer with Drabkin's reagent in the reference and sample cells.

9.1.3 Measure the absorbance of Fisher Standards I, II and III.

9.1.4. Plot the absorbance against grams of Haemoglobin per 100 ml of whole blood

9.2 Acetylcholinesterase and Pseudocholinesterase

9.2.1 No standards are required. However, the precision of the method should be checked by analysing commercial control sera of low and normal pseudocholinesterase activity (Level I and II) each day that samples are run.

10.0 CALCULATIONS

10.1 Calculations - Haemoglobin

The concentration of Haemoglobin (Hb) in grams per 100 mL is given by:

C (g/100 mL) = $R \times A$

C = Concentration of unknown g/100 mL

R = Instrument response $\frac{\text{g/100 mL}}{\text{Absorbance}}$

A = Absorbance reading of unknown

10.2 Calculations - Acetylcholinesterase

10.2.1 Measure the change in absorbance (ΔA) over five minutes from the chart recording over the linear part of the reaction (see Appendix).

$\Delta A = A_F - A_I$

A_I = initial absorbance reading

A_F = final absorbance reading

Rate/min = $A/5$ min.

10.2.2 The activity of acetylcholinesterase in micromoles litre^{-1} minute^{-1} (g Hb dL^{-1})$^{-1}$) at 25 ± 0.1°C is:

$$\text{ACHE Activity} = \frac{\text{Sample Dilution}}{0.0136} \times \frac{\text{Rate}}{(\text{g Hb dL}^{-1})}$$

Sample Dilution = 600

Molar Absorbtivity = .0136 L umole^{-1} cm^{-1}

Rate = Absorbance Change/min.

Note 1: All samples are analysed in duplicate and the average activity calculated.

2: The activity of acetylcholinesterase can be converted to international enzyme units (IU/L) of umoles of substrate hydrolysed per litre per min. at $37^{O}C$ by:

IU/L ACHE = Activity (at 25 ± $0.1^{O}C$) x 1.74

10.3 Calculations - Pseudocholinesterase

The activity of pseudocholinesterase in umoles minute^{-1} litre^{-1} at 25 ± $0.1^{O}C$ is given by:

$$CHE_{activity} = \frac{1000}{.165} \times \Delta A$$

0.165 = the absorbance change corresponding to the hydrolysis of 0.025 umoles/mL of benzoylcholine for a 1 cm. cell

ΔA^* = change in absorbance over five minutes.

*For a linear change in absorbance (See Appendix I).

Note 1: All samples are analysed in duplicate and the average activity calculated.

2: The activity of pseudocholinesterase can be converted to international enzyme units (IU/L) of umoles of substrate hydrolysed per litre per min. at $37^{O}C$ by:

IU/L CHE = Activity (at 25 ± $0.1^{O}C$) x 1.74

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APPENDIX I

Figure 1 - Acetylcholinesterase

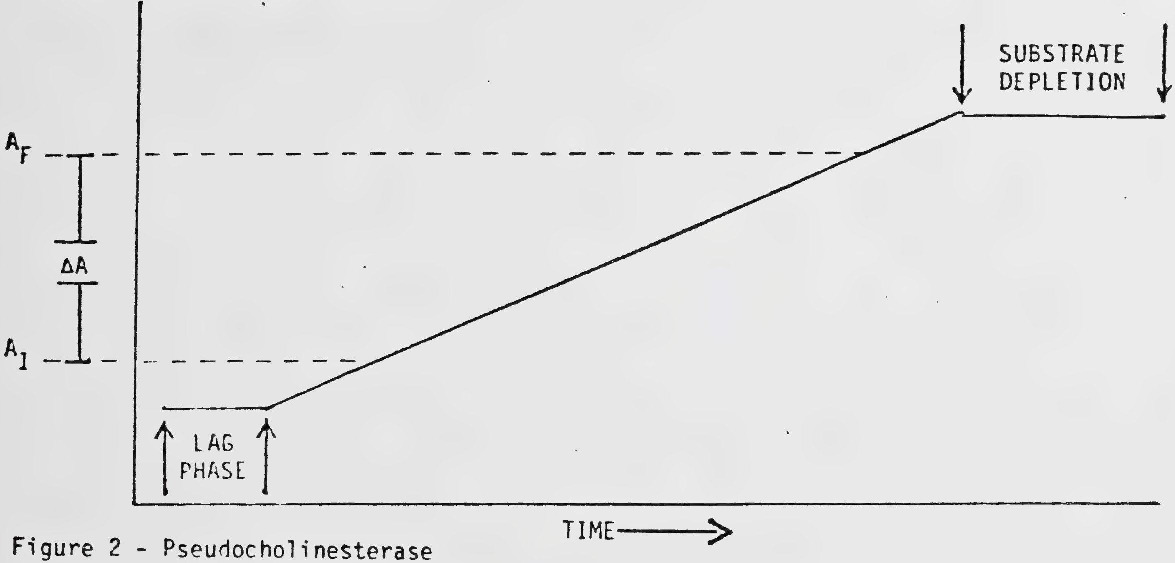
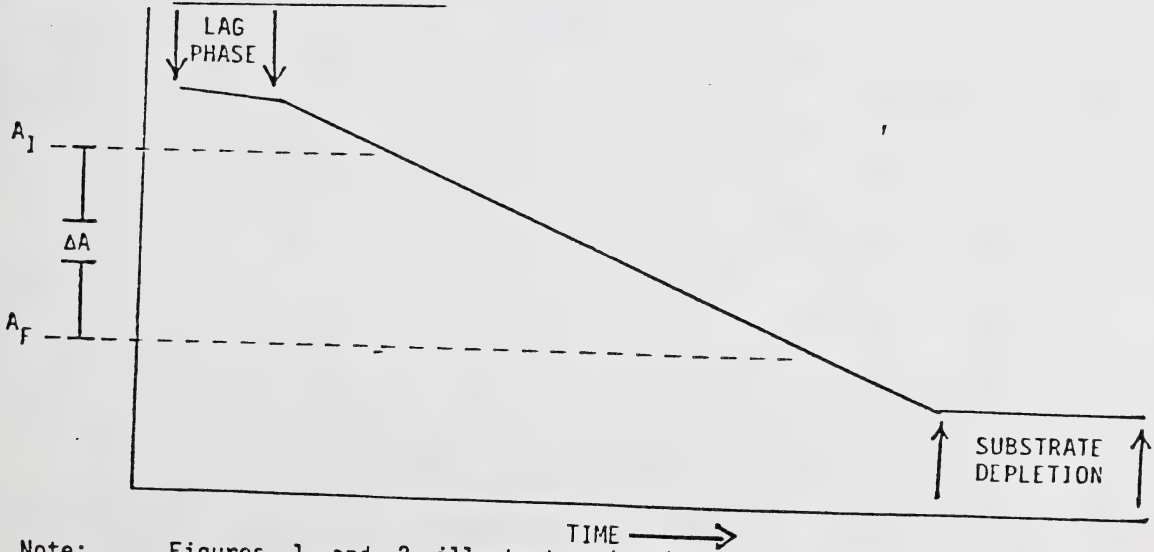


Figure 2 - Pseudocholinesterase



Note: Figures 1 and 2 illustrate absorbance as a function of time for acetylcholinesterase and pseudocholinesterase. The change in the absorbance is measured during the time where the curve is linear. The time frame for this method is normally five minutes but may be shortened (e.g. three minutes) to accomodate elevated enzyme activity, in which case the absorbance change would be measured over a reduced time frame.

Effects of Roadside Application of Carbofuran on Nesting Brewer´s Blackbirds

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Submitted to:
Crop Protection Branch
Alberta Agriculture

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Pecan Resources Inc.
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EXECUTIVE SUMMARY

This study examined the effects on nesting success and nestling growth of Brewer´s Blackbirds exposed to Furadan 480 Flowable applied by ground spray vehicle. Effects from dermal and inhalation exposure only were examined; oral exposure from ingestion of treated insects was low due to foraging locations. Two study areas were used, one near Drumheller and one near Lacombe.

The insecticide was applied to nests during the incubation and nestling stages. Eighteen nests were treated during the incubation stage, with 13 untreated incubation-stage nests monitored as controls. About one-quarter of nests containing eggs were directly exposed to the insecticide due to flushing of adults in advance of the vehicle. No effects of the treatment on egg- or hatching-stage survival, hatching rates or abandonment of nests by adults was seen.

Twenty-six additional nests were treated during the nestling stage and compared to 41 untreated nests. The daily mortality rate of nestlings at one of these sites was significantly higher than in untreated nests. This may reflect predation rather than nestling death. No dead nestlings were found. The overall survival rate from egg stage to fledging was similar in treated and untreated nests.

Carbofuran residues on nestlings collected between 13 and 33 hrs post-spray were low to nondetectable, indicating that little direct dermal exposure had occurred. The highest amount found was 0.24 ppm. Residues may have been reduced by rainfall between the time of treatment and collection.

Nestling growth rates, as measured by daily weight gain and growth of the tarso-metatarsal, were compared in treated and control nests and found to be similar at one site; at the other, differences may have been due to dampness of nests resulting from frequent precipitation.

INTRODUCTION

In 1985, Alberta Agriculture commissioned a preliminary investigation into the effects of grasshopper insecticide spraying of roadsides on bird nesting success. Nests of the most abundant species situated along roadsides were monitored for mortality, abandonment, hatching and nestling success and nestling growth. The study found two possible causes for concern: a) three dead nestlings within one nest having abnormal postures characteristic of cholinesterase inhibition; and b) significantly lower egg survival, hatching and nestling survival rates in the treated nests (Horstman 1985).

Due to small sample sizes and the fact that spraying was conducted by farmers as part of their normal operation (hence treatment was not randomized), the present study was planned. This study uses nesting Brewer´s Blackbird (Euphagus cyanocephalus) colonies for monitoring the effects of ground application of carbofuran (Furadan 480 Flowable).

This study was funded by Alberta Agriculture.

DESCRIPTION OF STUDY SITES

Two study areas were employed in order to repeat the experimental spraying during the second nesting period (late June-early July). The sites were selected on the basis of a) low grasshopper density (in order to avoid insecticide spraying by farmers), b) similarity of habitat type to areas within the province experiencing high grasshopper infestations, c) sufficient populations of Brewer´s Blackbirds for the study, and d) homogeneous terrain and general habitat appearance within an area of sufficient size for the study.

Site 1, used in June during the first nesting period of the Brewer´s Blackbird, is located within the mixed grass ecoregion (Strong and Leggat 1981) in the Morin-Three Hills area. This area contains mixed pasture and cropland. Its southern boundary was approximately 10 km from an area of heavy grasshopper density.

Site 2, situated within the groveland subregion of the aspen parkland between Alix and Lacombe, was used in late June-mid-July. This area is mostly cropland and relatively distant from severe grasshopper infestations; however, other areas within this same ecoregion were expected to have severe infestations in 1986 (D.Johnson, 1986).

Most of the blackbird colonies selected were located on roadsides adjacent to local roads; a few at Site 2 were situated on a joint highway and railroad right-of-way. The roadside vegetation at both sites consisted of a mixture of seeded domestic grasses, primarily brome, and clover. Shrubs such as Rosa, Symphoricarpos, and Salix spp. were occasionally present on roadsides at both sites, and some nests were situated in these species (particularly Rosa) to a height of about 0.5m. The majority of nests, however, were on the ground, often in a dense clump of clover or grass. No other roadside spraying of the nesting sites was conducted in 1986 prior to or during the study.

METHODS

Field Monitoring

Searches for Brewer´s Blackbird nests along roadsides were conducted from 24 May-3 June at Site 1 and 24 June-2 July at Site 2. A wooden stake was placed 4 m from each nest located. Nests were visited briefly once daily, usually by the same worker. Nests were not disturbed during rain. Nestlings were color-coded with felt pen on the back and legs. Nestling weights were recorded daily to the nearest 0.5 g with Pesola and Jim 50- and 100-g spring scales. The length of the tarso-metatarsus was measured to the nearest 0.1 mm with vernier calipers as described by Godfrey (1966). Disposable plastic gloves were used when handling nestlings, eggs or surrounding vegetation. The presence or absence of the adult pair, adult foraging behavior, appearance of the nestling, predator sign and other relevant information were noted.

Study Design

Sixteen colonies were identified and monitored at Site 1. External factors such as predation, flooding, cowbird parasitism and use of insecticides on adjacent fields (as determined by interviews with landowners) reduced the number of useable colonies to 10 by the time of treatment. At Site 2, 14

colonies were initially monitored, of which 10 were still intact by the time of spraying.

Colonies were randomly assigned to either the spray treatment or no treatment (control). In three instances, landowners adjacent to designated spray colonies did not approve of the planned roadside spraying. In these cases the colonies were redesignated control colonies and other colonies were randomly selected to replace them.

Sprayed and control colonies which were closest together were paired for data analysis of nestling growth. This was done partly to address the variability introduced by localized rainshowers and hailstorms which occurred in parts of the study area. Paired colonies were monitored by the same worker (with the exception of holidays). Figure 1 shows the locations of paired colonies (blocks) at Site 1. The average intrapair distance was 7.2 km (4.5 mi); the average distance between a colony and its nearest non-pair neighbor was 20.4 km (12.8 mi). Colonies at Site 2 were not paired for data analysis.

Insecticide Application

Nests at Site 1 were sprayed with Furadan 480F when the majority of nests were in the egg stage and again 10 days later when the majority were in the nestling stage. Due to inclement weather at Site 2, nests were sprayed only in the nestling stage. Table 1 gives the details and dates of the spraying regime. Furadan 480F was applied with a truck-mounted hydraulic boom sprayer. At Site 1 the boom was equipped with 80 flat fan nozzle tips and an end off-center (o.c.) nozzle while at Site 2 it was equipped with 3/4 o.c. nozzles only. Boom height was maintained at approximately 1/2 m above the roadside, with sections adjusted to follow the contour of the ditch; the end section was raised when necessary to spray nests in shrubs. Travel speed and pressure were set to deliver 10-11 gal/ac at 275 kPa. Spray was applied for a distance of 0.4 km (0.25 mi) along the right-of-way on both sides of a colony and for the same distance on the opposite side of the road.

Residue Analysis

Sprayed nestlings were collected for body carbofuran residue analysis within 13-35 hours after the nestling-stage spray application at Site 1. Birds were

killed by cervical dislocation and bodies were stored in dry ice for 35 days followed by storage at -70°C until analysis (3 weeks for 10 samples; 17 weeks for 5 samples). Carbofuran residues were analyzed from whole-body extracts (minus head) including feathers. Samples were prepared following a modification of the method of Cook et al.(1969) and analyzed by GC/MS by Envirotest Laboratories, Edmonton.

RESULTS

External Factors and Behavior

Weather

Precipitation was relatvely abundant at both the June and July sites. At Site 1, approximately one-third of all days during the study period had some precipitation, including localized rainshowers which did not always cover the entire study area and two hailstorms which occurred during the nestling stage in part of the area. Minimum daily temperatures were often as low as 8 C. At Site 2 rain also occurred on approximately one-third of all days. A heavy rainstorm which lasted 2 1/2 days occurred during the late nestling stage. This storm probably caused some nestling mortality (see below).

Application of insecticide was postponed due to the inclement weather at Sites 1 and 2. Incubation-stage spraying at Site 2 was omitted for this reason. Some spraying was carried out despite the threat of rain. Table 2 shows the chronology of the insecticide applications and subsequent precipitation. Light rain fell in much of the Site 1 area within 6 hrs after both the first and second insecticide applications. Rain fell within 12-16 hrs of spraying at all Site 2 colonies. The maximum daily temperature on all days during which spraying was conducted was 20-21° C.

Effect of Spray Vehicle

The approach of the spray truck caused some adult females to flush from their nests immediately ahead of the boom; in some cases the adult was sprayed while passing under the boom. One-quarter of all females were probably sprayed during the egg stage application. Fewer females were sprayed during the nestling stage application (see Discussion).

Foraging Behavior

Sprayed insects comprised only a small portion of nestling diets at either site. Contrary to expectations based on the previous year´s study, which found much foraging by adult females for insects during the nestling stage on the rights-of-way adjacent and opposite to their nests, this study found most foraging in nearby summerfallow fields, crops (unsprayed; see Methods) and around livestock, if present nearby. Gizzard contents of adults and fledglings collected from one of the control colonies approximately 2 weeks post-fledging found a diet high in grasshoppers (Dore and Johnson 1987; see Appendix); however, females often carried soft-bodied insects to nestlings.

Nesting Survival

The number of active nests per colony at the time of first treatment averaged 4.5 and 1.7 at Sites 1 and 2, respectively. Site 2 nesting may have represented a second brood by a small proportion of females and/or renesting by individuals following an unsuccessful first attempt.

Figure 2 shows the age of all treated eggs and nestlings. Nesting was not synchronous at either site. Hatching dates for Site 1 nests ranged from 31 May-27 June, with the mode on 6 June. Due to this asynchrony and a delay in the first treatment due to weather, only 18% (6) of 33 treated nests at Site 1 were treated twice (once in both egg and nestling stages) as originally planned for all nests. Data from these nests were pooled with other egg-stage treated nests for egg survival and hatching rate determination but were examined separately for nestling success.

Egg Survival

A total of 92 eggs in 18 nests were sprayed at Site 1 while 63 eggs in 13 nests were used as controls over the same period (Table 3). Clutch size at Site 1 was 4.7 ± 0.8 in control nests and 5.0 ± 0.9 in treated nests. Treated eggs ranged in age from days 5-14 of incubation at the time of treatment (the incubation period lasting generally 14 days), with the median age at treatment day 9 (Figure 2). The age distribution of control eggs used for comparison of egg success was similar, the median age at the 6 June treatment also being day 9.

The daily mortality rate, or number of eggs lost per egg-day (one egg for one day) between the date of treatment and the onset of the hatching period was calculated by the method of Mayfield (1975). A post-spray loss of 3% (12 eggs/451 egg-days) occurred in the treated nests and 1% (5/380) in control nests. Compared by the method of Johnson (1979), these rates did not differ ($Z = 1.41$; $P>0.05$; Table 3).

Hatching Rate and Survival

The hatching period lasted 2-3 days in most nests. Daily mortality of eggs and/or newly hatched nestlings during this stage was 6% (13/205 egg- or nestling-days) in treated and 4% (18/453) in control nests. This difference was not significant (Table 3).

The hatching rate, or ratio of nestlings at the end of the hatching period to eggs present at the onset of hatching, was 84% in control nests. This represents rates in control nests before and after the 6 June treatment date, since these rates did not differ [X2(1df) = 0.84, $P>0.05$; Table 4]. Site 2 nest hatching rates in July were similar Site 1 control nest rates [X2(1df) = 1.3; $P>0.05$; Table 4].

Treated nests at Site 1 had a hatching rate of 76%. Nests which were sprayed within 24 hours of hatching (n=6) were examined separately because of the possibility that these nestlings might have encountered a greater amount of carbofuran than the others during the pipping (eggshell breaking) process. However, the rate for these eggs was found to be similar (79%; 19/24) and was therefore combined with the treated nest data, yielding an overall hatching rate of 77%, which did not differ significantly from that of the control eggs [X2(1df) = 1.69; $P>0.05$; Table 4].

Nestling Survival

A total of 13 nests containing 49 nestlings were treated during one of the two insecticide applications at Site 1. An additional 6 nests were treated during both egg and nestling stages. Ages of the sprayed nestlings ranged from 1-10 days (day 10 being the day at which most nestlings fledged), with a large peak (43%) at day 2 (Figure 2). Thirty untreated nests containing 124 nestlings served as controls during this period.

During a total of 168 post-spray nestling-days (one nestling for one day, as per Mayfield 1975) in treated nests at Site 1, 13 nestlings were lost, giving a daily mortality rate of 8%. The 6 nests which were sprayed in both stages also experienced a mortality rate of 8% (8 nestlings/95.5 post-spray nestling-days); therefore the data were pooled.

Control nests at Site 1 experienced a 2% nestling mortality rate (15/781 nestling-days), which was significantly lower than the 8% treated nestling mortality rate ($Z = 3.48$; $P<0.05$). Although the average number of nests in control colonies exceeded that in treated colonies during this stage, there was no correlation between colony size and mortality ($t=-.28$; $P>0.05$).

At Site 2, 7 nests containing 28 nestlings were treated and 11 nests containing 46 nestlings served as controls. The average age of treated nestlings at the time of treatment was day 6, with ages ranging from days 2-8 (Figure 2). Because of a heavy 2-day rainstorm on 17-18 July (7 days after treatment) which immediately preceded the death of 12 nestlings (2 treated, 10 control), only the 7-day period between treatment and storm were used for calculation of nestling success. During this period treated nests experienced a mortality rate of 6% (7/121.5 nestling-days) and control nests, 5% (12/250.5). These rates were not significantly different ($Z = 0.38$; $P>0.05$).

Nestling mortality was the same in Site 1 and 2 treated nests ($Z = 0.81$; $P>0.05$). Site 2 control nests experienced higher mortality rates than Site 1 ($Z= 2.03$; $0.05>P>0.01$; Table 3).

Overall Survival Rates

The daily survival rate (1- the daily mortality rate) for each nesting stage is shown in Table 3. Combined over the 3 stages, they gave overall daily survival rates of 84% and 93% for treated and control nests, respectively, with 95% confidence limits (± 2 S.E.) of 76 - 92% and 89 - 97% respectively. Thus no differences in mortality were seen.

Predation and Abandonment

The nesting survival rates above include both predation and abandonment. Abandonment as judged by apparently undisturbed eggs or nestlings with no

female, averaged 8% (1/13) and 0% in incubation-stage control and treated nests, respectively at Site 1 (Table 5). During the nestling stage, no abandonment of treated nests occurred, while 3% (1/30) of control nests were abandoned. At Site 2, no abandonment of nestling-stage nests occurred post-treatment. These are a minimum estimate, since the disappearance of entire nests of eggs and nestlings which occurred occasionally could have been preceded by abandonment. Whole nest predation occurred in 11% (2/18) of treated nests and 0% of control nests during incubation and in 21% (4/19) of treated and 0% of control nests during the nestling stage at Site 1 (Table 5). Only 1-4 incidents of whole-nest predation and/or abandonment occurred in either sprayed or control nests.

Growth Rates

Site 1

The average daily growth of nestlings from hatching through day 9 at Site 1 is shown in Figure 3 and Table 6. The relationship between weight and age and between length of the tarso-metatarsal and age during this period was determined by multiple regression. A straight line describes these relationships well for both treated and untreated birds (Figure 4; Table 7).

Using the 5 paired treatment and control colonies at Site 1 (Figure 1), a paired samples t-test (Snedecor and Cochran, 1980, p.86) was conducted to determine whether the average daily weight gain or metatarsal increase per nestling differed between treated vs control colonies over the first 9 days post-hatching (Table 8). No significant differences were found ($t = 0.68$, 4 df, $P>0.05$, daily weight gain; $t = 1.37$, 4 df, $P>0.05$, daily tarso-metatarsal increment).

A further test was conducted to detect possible early changes in growth rates which may have been concealed by later resumption of normal rates. Mean weight gains of those treated and control nestlings that were 1 or 2 days old at the time of spray treatment were compared over the first 3 days post-treatment. Nineteen nestlings from 4 treated colonies and 47 nestlings from 4 control colonies were compared by means of Student's t-test. The mean 72-hr gains of 12.8 ± 3.3 g for the controls and 14.5 ± 4.0 g for the treated nestlings were similar ($t = 1.71$; $P>0.05$).

Site 2

Growth of Site 2 nestlings is shown in Figure 3 and Table 6. The regressions of control nestling body weights and tarso-metatarsal lengths on age were linear (Figure 4, Table 7) and similar to those of the control nestlings at Site 1 as seen in an analysis of covariance (age as covariant; F = 0.00003; P>0.05).

However, the regressions for the treated nestlings, while still linear (Figure 4, Table 7), had non-parallel slopes to those of the control nestlings. This was due to lower and occasionally negative growth rates in the former. Weight declines exceeding 1 g occurred in 45% (13/28) of the treated and 6% (2/34) of the control nestlings in the week between insecticide application and the heavy rainstorm. Most (75%) of the nests in which these weight declines occurred were on the ground, which was wet or damp during much of this period. Some nests were also wet at this time. Since more treated than control nests were situated on the ground (71%, 5/7 treated; 33%, 3/9 control), a possible difference due to ground vs shrub location was eliminated by comparing data from ground nests only (the small number of treated shrub nests precluded 2-way anova). Daily weight gains of 3-8-day old nestlings were higher than those of similar-aged treated nestlings [5.3 ± 2.4 g (n = 30) control; 3.9 ± 3.0 (n = 44) treated nestlings; t(72) = 2.02, 0.05> P>0.01]. Mean daily tarso-metatarsal growth was also higher in the control nestlings [3.48 ± 1.4 mm, control; 2.69 ± 1.4 mm, treated nestlings; t(98) = 2.77, P<0.01]. Because nest dampness was not recorded in some cases, it was not possible to examine this as a contributing factor to the growth rate differences.

Carbofuran Residues

Six of the 14 treated nestlings assayed had detectable levels (>0.01 ppm) of carbofuran, ranging from 0.24 to 0.01 ppm wet weight (Table 9). No 3-hydroxy carbofuran was detected. The highest carbofuran level occurred in an older nestling which had fledged within 30 hours after spraying and was found 5 m

from the nest. Since the bird appeared active and healthy, it probably had travelled this distance on the ground (newly fledged blackbirds being unable to fly), contacting sprayed vegetation and soil in the process.

Physical and Behavioral Abnormalities

One nestling appeared highly blistered shortly after the spray application. This was judged from photographs to be due to a ruptured air sac resulting from some externally applied pressure and not related to chemical exposure (Dr.J. Hansen, pers.comm.)

Unusual adult female behavior was observed at a treated colony between 1700-1900 hours, approximately 10 hours after spraying. Females perched on fenceposts or overhead wires, returning to their nests only occasionally and for unusually short durations. They appeared to be less afraid of the observer than usual; as a result, 5 previously unidentified nests were located within an hour, the amount of time often required to locate 1 or 2 nests. This behavior was not seen again.

DISCUSSION

This study evaluated direct dermal or respiratory toxicity from ground roadside spraying. Adults foraged for insects primarily in fields rather than on roadsides during the nestling stage; hence this study did not investigate oral toxicity from ingestion of treated insects. The oral toxicity of carbofuran to "wild birds" is reported to be much higher than the dermal: LD50 = 0.00042 g/kg oral and 0.10 g/kg dermal (Environment Canada 1984).

About one-quarter of the egg-stage nests were sprayed when the female flushed ahead of the spray boom; the rest three-quarters probably remained covered by brooding females. Thus direct exposure of the eggs may have been minimized by the behavior of the female.

Nests with nestlings, on the other hand, were more likely to be exposed to the spray, as females spent less than 1 out of every 5 minutes at the nest feeding the young and were more often away from their nests during the spraying. Nevertheless, the residues found suggest that nestlings either received or retained little pesticide. Several factors may have contributed to the low residues found. Rain may have rinsed unabsorbed chemical from the skin, feathers and nest material, as carbofuran is highly water-soluble (700mg/L; NRCC 1979). Nevertheless, as absorption of Furadan by the insect cuticle takes place within 1-6 hours (M. Dolinski, pers. comm.), it is likely that some of the spray droplets that reached the nestlings would have been absorbed by the skin and feathers prior to the precipitation. The fact that the highest residue occurred on a nestling that had left its nest approximately 24 hrs post-treatment and travelled 5 m through the roadside ditch suggests that the nest environment, with its vegetative cover and crowded conditions, may afford considerable protection from sprayed chemicals, whereas fledged birds which leave the nest around the time of application may be more exposed through contact with coated vegetation and soil.

Temperature may have influenced the amount of effect which occurred. The maximum daytime temperatures during the spraying, 20-21°C, are in the temperature range found by Rattner and Camardese (1985) to have the least effect on cholinesterase activity levels of Japanese quail fed organophosphate

insecticides. For the same oral dose, a 15% decrease in ChE activity occurred at 25°C and a 41% decrease at 37°C.

The treatment did not cause adult mortality or abandonment of nests. The higher nestling daily mortality rate in one of the two study areas is difficult to explain, as losses could represent predation or discarded sick or dead nestlings. Dead nestlings were not in evidence. As growth rates at Site 1 showed no treatment effect, it is doubtful that sickness was the cause.

The effects of an aerial application of this insecticide could not be predicted from the results of this study because of the difference in droplet size, which in turn could affect inhalation if not dermal toxicity. In addition, the area of treated insects would be larger.

ACKNOWLEDGEMENTS

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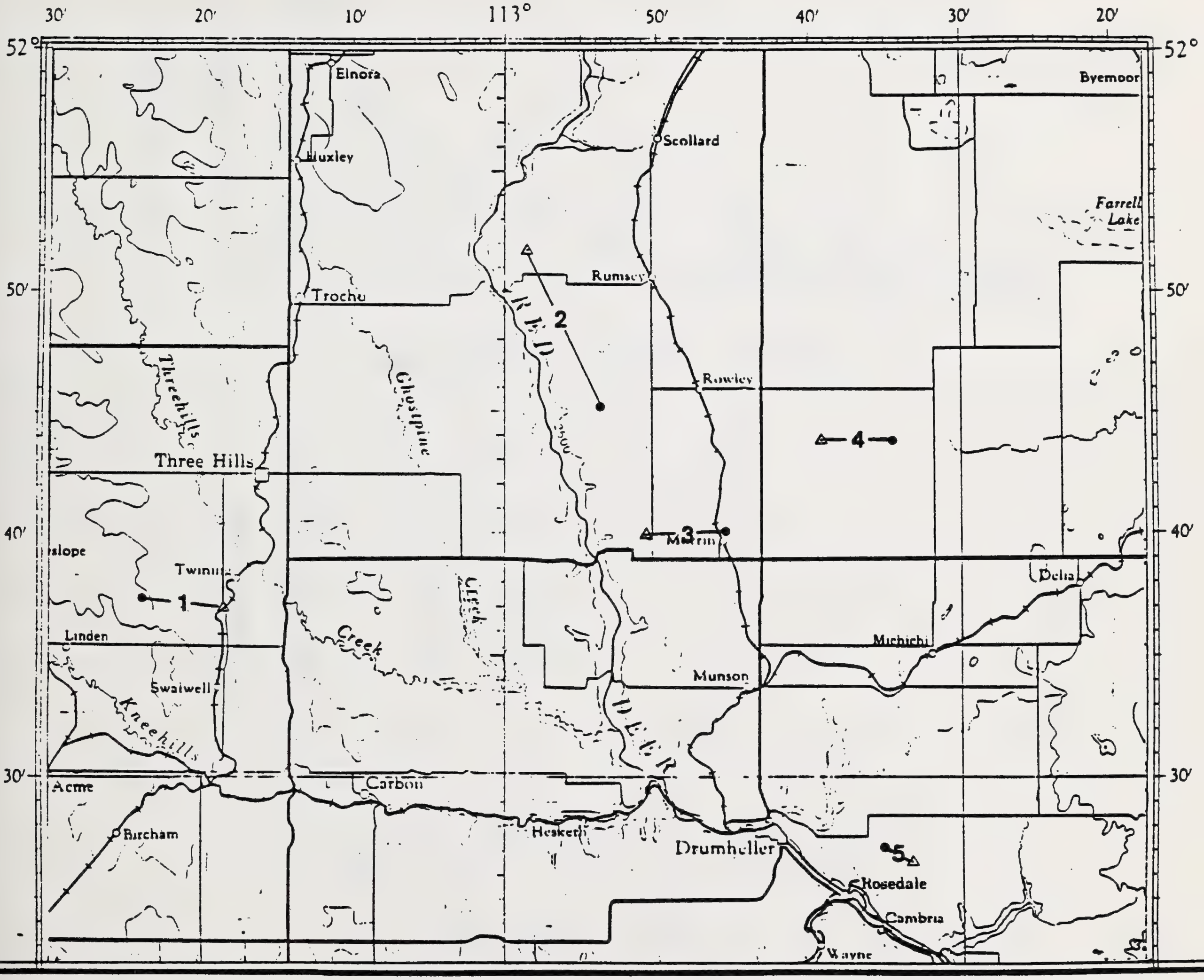
Figure 1. Location of paired colonies at Site 1



Scale: 1:500,000 1cm = 5km

Legend:
3 - Block
● - Control
△ - Treated

Map Reference: Canada Department of Energy, Mines and Resources
Surveys and mapping Branch
Banff - Bassano, N.T.S. No. 82 N.E.

FIGURE 2. AGE OF EGGS AND NESTLINGS AT TREATMENT
H = hatching

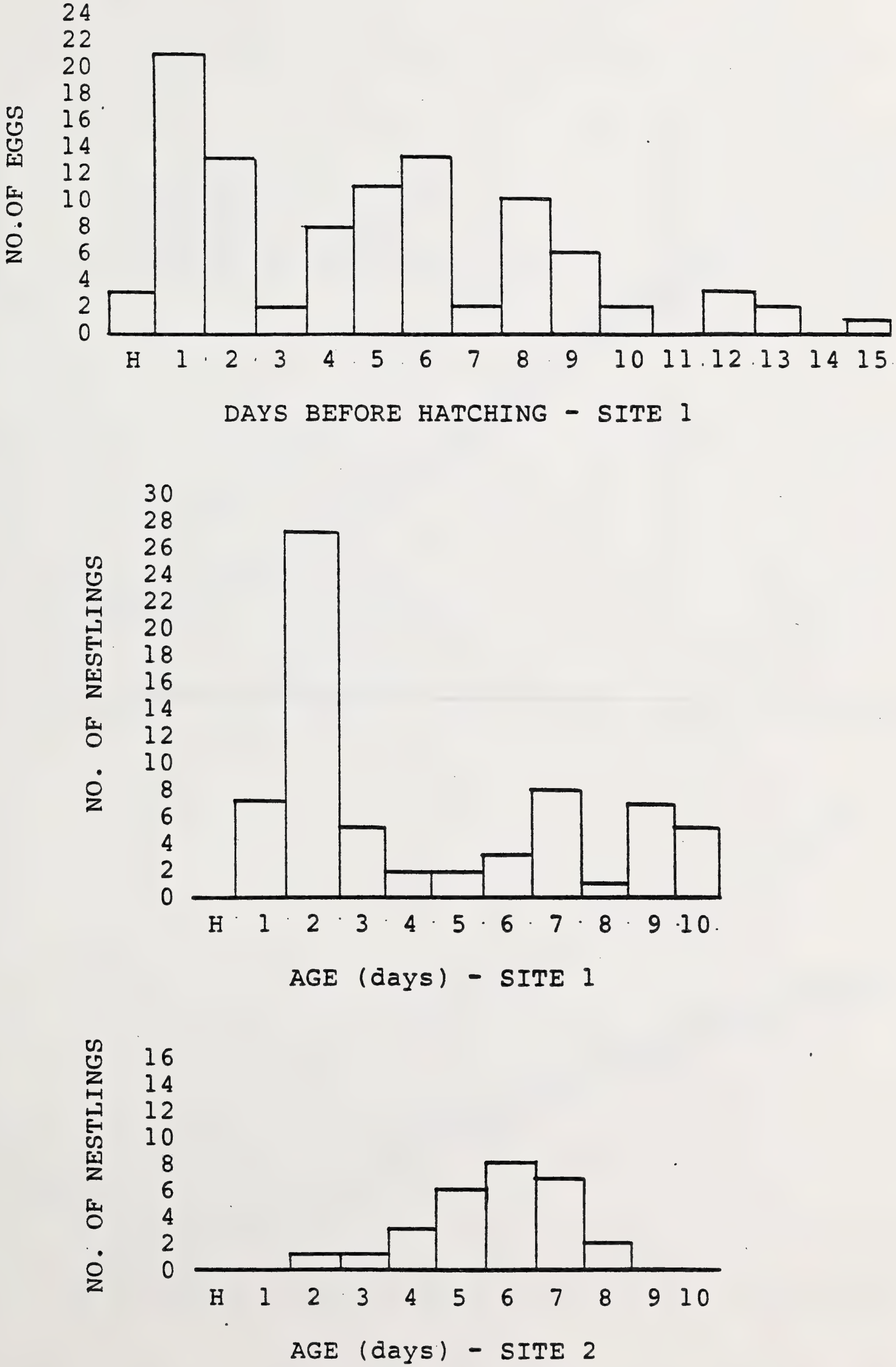


Figure 3. Growth of treated and control nestlings at Sites 1 and 2 (Site 1 and 2 controls pooled; P>0.05)

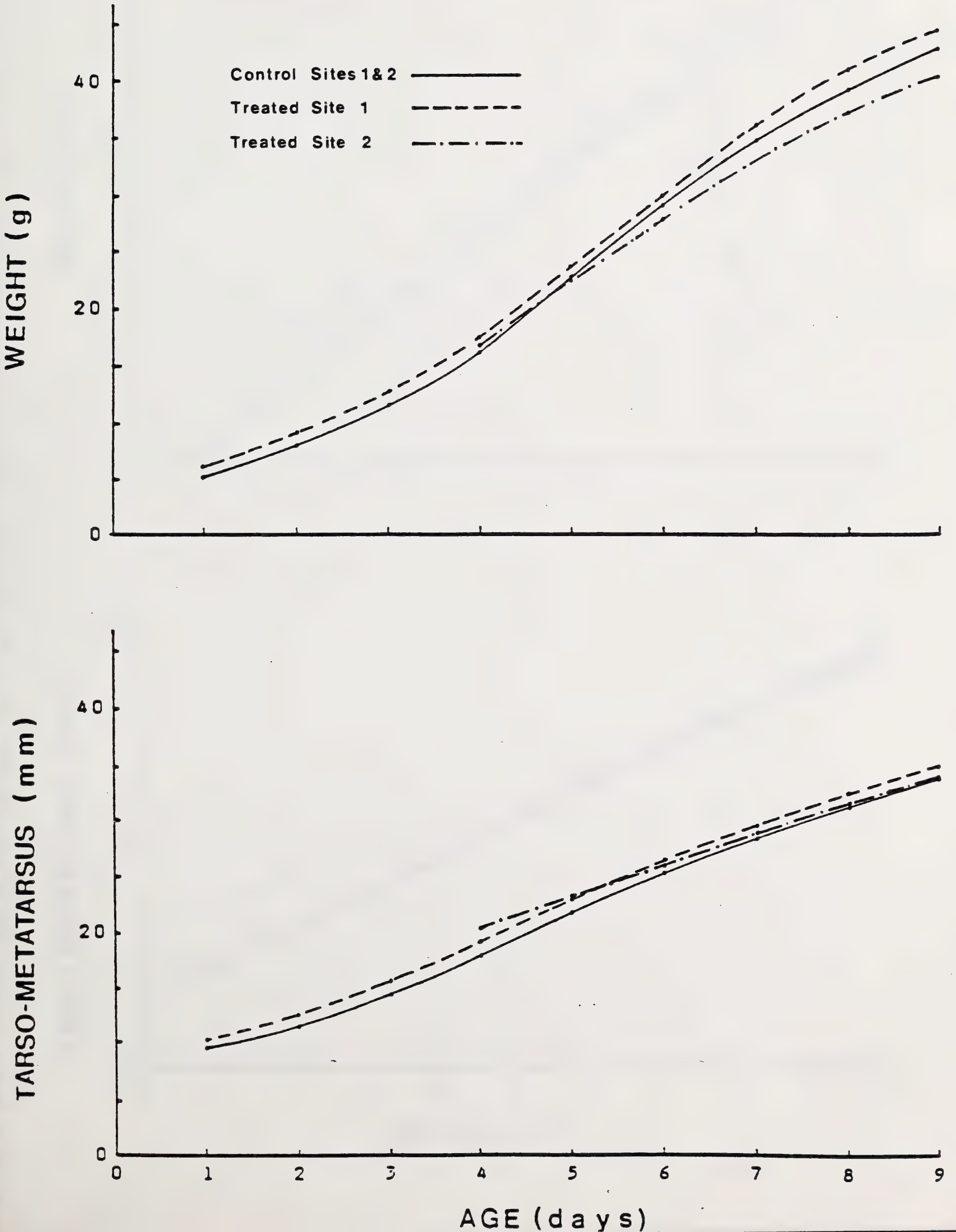


Figure 4. Regression of weight and tarso-metatarsal length on age of treated and control nestlings at Sites 1 and 2

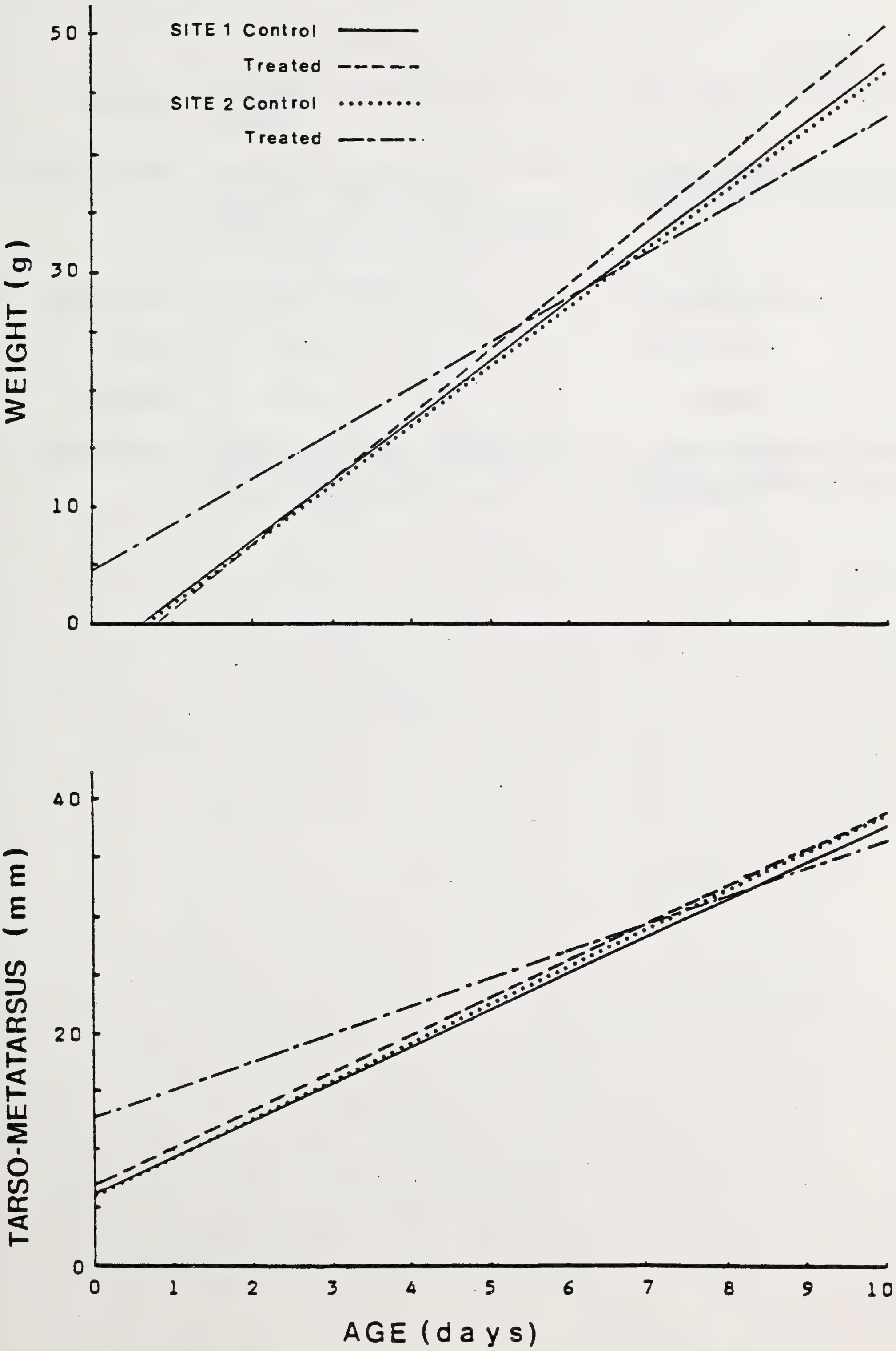


Table 1. Spray treatment

	Site 1	Site 2
location:	Drumheller-Three Hills area	Lacombe-Alix area
date & time:	eggs: 5 June 0700-1200h; 6 June 0630-1630h nestlings: 15 June 0930-1430h; 16 June 0630-0730h	nestlings: 9 July 1000-1300h; 10 July 1300-1700h
chemical:	Furadan 480F	Furadan 480F
rate:	275 mL/ac	275 mL/ac
volume:	10 gal/ac	11 gal/ac
equipment:	truck-mounted hydraulic boom sprayer with 80 flat fan nozzle tip	truck-mounted hydraulic boom sprayer with 3/4 o.c nozzles

Table 2. Time between insecticide application and precipitation at Sites 1 and 2

Site 1

	first application		second application	
colony #	time between treatment and rain (hrs)	intensity	time between treatment and rain (hrs)	intensity
510	5	drizzle	5	very light
210	5	drizzle	-	none
220	1.5	light	6	?
860	1.5	light	-	none
120	1.5	drizzle	6	?

Site 2

nestling stage treatment

colony #	time between treatment and rain (hrs)	intensity
123	16	moderate
222	12	light
860	12	light
380	12	light
320	12	light

Table 3. Nest success and mortality in treated and control nests at Sites 1 and 2

Egg stage	Treated	Control
colonies	8	5
nests	18	13
eggs	92	63
egg-days post-spray	451	380
eggs lost post-spray	12	5
daily mortality*	.027	.013
daily survival (%)	97	99

treated vs control mortality rate:
Z= 1.41; P>0.05

Hatching stage	Treated	Control
egg/nestling-days	205	453
eggs/nestlings lost	13	18
daily mortality*	.063	.039
daily survival (%)	94	96

treated vs control mortality rate:
Z= 1.20; P>0.05

Nestling stage	Site 1		Site 2	
	Treated	Control	Treated	Control
colonies	7	6	5	7
nests	19	30	7	11
nestling-days	263.5	781	121.5	250.5
nestling losses	21	15	7	12
daily mortality*	.079	.019	.058	.048
daily survival (%)	92	98	94	95

Site 1 treated vs control mortality rate: Z =3.48; P<0.01
Site 2 treated vs control mortality rate: Z= 0.38; P>0.05
Site 1 vs 2 treated mortality rates: Z= 0.81; P>0.05
Site 1 vs 2 control mortality rates: Z= 2.03; 0.05>P>0.01

	Treated	Control
Overall daily survival rate (%) (egg x hatching x nestling stages)	84	93
95% confidence limits (%)	76-92	89-97

* no. losses/no. egg- or nestling-days

Table 4. Chi-square comparison of hatching rates

A. Comparison of pre-and post-treatment date hatching rates in Site 1 control nests

	pre-6 June	post-6 June
#eggs pre-hatching	71	47
#nestlings post-hatching	59	40
hatching success (%)	83	85

X2(1df)=0.84; P>0.05

B. Comparison of hatching rates in Site 1 and 2 control nests

	Site 1	Site 2
#eggs prehatching	118	57
#nestlings post-hatching	99	50
hatching success (%)	84	88

X2(1df)= 0.44; P>0.05

C. Comparison of Site 1 hatching rates in treated and control nests

	Treated	Control
#eggs prehatching	109	118
#nestlings post-hatching	87	99
hatching success (%)	77	84

X2 (1df)= 1.69; P>0.05

Table 5. Abandonment and whole nest predation during the post-treatment period at Sites 1 and 2

	abandonment		whole nest predation	
	incubation stage % (n)	nestling stage % (n)	incubation stage % (n)	nestling stage % (n)
Site 1				
control	8 (1/13)	3 (1/30)	0	0
treated	0	0	11(2/18)	21(4/19)
Site 2				
control	n/a	0	n/a	18 (2/11)
treated	n/a	0	n/a	14 (1/7)

Table 6. Mean daily body weight and length of tarso-metatarsal in treated and control nestlings, Sites 1 and 2

Tarso-metatarsal (mm)

	Site 1		Site 2	
day	control (n)	treated (n)	control (n)	treated (n)
1	9.8±0.7 (127)	10.4±0.3 (3)	10.3±0.9 (42)	
2	11.9±1.0 (120)	12.2±1.9 (15)	12.2±1.2 (39)	
3	14.8±1.5 (111)	15.6±2.4 (30)	15.4±1.5 (36)	15.2±0.0 (1)
4	18.5±2.2 (101)	19.3±2.4 (30)	18.8±1.9 (36)	20.7±1.8 (3)
5	22.5±2.2 (101)	23.4±2.6 (30)	22.5±2.5 (36)	23.5±1.8 (6)
6	25.8±2.5 (94)	27.4±2.5 (29)	27.0±2.1 (32)	27.1±2.0 (15)
7	29.6±2.3 (79)	30.9±2.2 (31)	30.7±2.6 (27)	29.8±2.4 (22)
8	32.5±2.0 (65)	33.5±1.6 (31)	33.6±2.1 (24)	32.7±2.7 (25)
9	34.4±2.1 (61)	35.4±1.8 (34)	35.5±1.6 (21)	34.6±3.1 (22)

Weight (g)

	Site 1		Site 2	
day	control (n)	treated (n)	control (n)	treated (n)
1	4.8±0.8 (125)	6.0±0.0 (3)	4.8±0.9 (40)	
2	7.4±1.3 (117)	8.1±2.1 (15)	7.4±1.5 (39)	
3	11.6±2.1 (110)	12.9±3.2 (30)	11.6±1.9 (36)	8.5±0.0 (1)
4	16.7±2.9 (101)	17.6±3.7 (30)	16.2±2.6 (34)	17.3±3.0 (3)
5	22.9±3.5 (99)	22.7±5.6 (30)	21.8±3.8 (33)	20.8±2.6 (6)
6	28.5±4.3 (93)	29.0±3.8 (29)	29.3±4.4 (28)	28.3±5.0 (15)
7	34.1±4.5 (78)	36.4±4.6 (31)	35.0±5.4 (21)	32.9±6.2 (22)
8	39.4±4.7 (65)	40.6±3.7 (32)	40.9±3.4 (21)	36.9±7.6 (25)
9	42.8±4.7 (62)	44.6±4.5 (33)	43.8±4.2 (19)	40.0±7.3 (22)

Table 7. Regression equations for nestling growth vs age, Sites 1 and 2

	tarso-metatarsal length		weight	
	control	sprayed	control	sprayed
	SITE 1			
	Y=6.0 +3.2 X	Y=6.7 + 3.3X	Y=-1.8+5.0X	Y=-2.7+5.3X
r2	0.94	0.92	0.93	0.90
F	(1,873) 14106	(1,246) 2727	(1,873) 12450	(1,246) 2196
P	<0.01	<0.01	<0.01	<0.01
	SITE 2			
	Y=6.2 + 3.3X	Y=12.3 + 2.5X	Y=-2.1+ 5.0X	Y=4.9 + 3.9X
r2	0.94	0.67	0.92	0.45
F	(1,301) 4979	(1,108) 220	(1,301) 540	(1,108) 87
P	<0.01	<0.01	<0.01	<0.01

Table 8. Paired t-test of average daily growth, Site 1

Block	Av. daily wt gain per nestling (g)		Av. daily growth of tarso-metatarsal per nestling (mm)	
	control	treated	control	treated
1	5.4	5.2	3.45	2.97
2	5.3	4.9	3.66	3.30
3	4.7	5.4	3.52	3.34
4	5.0	4.8	3.36	3,53
5	5.1	4.4	3.23	3.26
	t(4df)=0.684 $p>0.05$		t(4df)=1.367 $p>0.05$	

Table 9. Carbofuran residues in sprayed nestlings

no.	age (days)	carbofuran (ppm wet wt)	3-OH carbofuran	time elapsed (hrs)
1	9	n.d.	n.d.	31
2	11	0.05	n.d.	35
3	10	0.24	n.d.	32
4	-	n.d.	n.d.	13
5	9	n.d.	n.d.	33
6	-	n.d.	n.d.	13
7	4	n.d.	n.d.	35
8	5	n.d.	n.d.	35
9**	9	0.02	n.d.	35
9**	11	0.02	n.d.	35
10	11	n.d.	n.d.	33
11	10	0.01	n.d.	33
12	8	0.01	n.d.	33
13	10	n.d.	n.d.	33
14	11	n.d.	n.d.	33

n.d.= not detected

* time between spraying and collection

**2 bird samples combined for analysis

#1-9 corrected for a 46% carbofuran recovery; #10-14 for 53% recovery. 3-OH recovery: 105%

APPENDIX

Gizzard contents of adult and juvenile (fledged) Brewer´s Blackbirds from a control colony at Site 1 (table from Dore and Johnson 1987)

Item	Total no. of occurrences	
	Females (n=18)	Males (n=11)
Grasshoppers	52.0	36.0
Orthoptera	0.9	0.5
Coleoptera	0.0	0.0
Others	0.0	0.1
Seeds	1.3	0.5
Spiders	0.1	0.0
Odonata	0.1	0.0

EFFECTS OF CARBOFURAN ON UNCONFINED SMALL MAMMAL POPULATIONS IN SOUTHERN ALBERTA

Provincial Grasshopper Taskforce
Government of Alberta

L.M. Brusnyk and D.A. Westworth

D.A. Westworth & Associates Ltd.
Edmonton, Alberta

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SUMMARY

In 1986, D.A. Westworth & Associates Ltd. initiated a pilot study designed to examine the effects of carbofuran on unconfined small mammal populations near Youngstown, Alberta. Four trapping grids were sampled, two of which were experimentally sprayed with carbofuran at the prescribed field rates. The remaining two grids were considered as control sites. Small mammals were live-trapped, tagged and released during a pre-spray trapping session designed to document pre-spray population levels on control and experimental grids. Immediately following the spraying program, a post-spray trapping session was initiated to determine any effects carbofuran applications might have on small mammal populations. In addition, 35 animals were collected for brain acetylcholinesterase and carbofuran residue determinations. A late summer trapping session was conducted in mid-August and was used to determine the effects of the spraying program on survival and reproductive characteristics of small mammal populations.

Peromyscus maniculatus was the most abundant small mammal species, accounting for approximately 80% of the 389 animals tagged. **Microtus pennsylvanicus** was the next most abundant species (19%) followed by **Mus musculus.** **P. maniculatus** populations were approximately three times higher in slough habitats than in open prairie types, however, they did not vary significantly between control and experimental grids of each habitat type or among the three trapping sessions, suggesting that carbofuran applications had no detectable effect on overall population density. Comparisons of various reproductive characteristics and brain acetylcholinesterase levels of the population also showed no obvious responses to carbofuran. However, survival rates for both **P. maniculatus** and **M. pennsylvanicus** were low on the experimental sites following exposure to carbofuran. Moreover, significant amounts of carbofuran residues were detected in the gastro-intestinal tracts of animals collected from the experimental grids sprayed with the insecticide but no residues were found in animals taken from the control sites.

The results of these initial investigations suggest that there were no large observable effects of carbofuran on absolute numbers of small mammals and for most of the other parameters examined. But the presence of significant amounts of carbofuran residues in the gastro-intestinal tracts of mice from

experimentally sprayed sites and the low observed survival rates suggests that a carbofuran-induced population response had occurred. It was suggested that there must have been some mortality of animals following the application of carbofuran to the experimental sites and that the relatively stable population levels observed among the three trapping sessions was a result of either young animals immigrating from adjacent habitats to occupy available habitat space left vacant by the death of a proportion of the resident population, an increase in natality rates over the summer period or a combination of both factors. It seems that carbofuran-contaminated foods were consumed by **P. maniculatus** but at levels that did not significantly affect overall population densities over the time period studied. Instead, the age distribution and possibly the social structure of the resident population may have been affected. Because of small population sizes, the need for information on the effects of repeated exposure on small mammals over a longer time period and a refinement in some of the study methodologies, further studies on confined small mammal populations are warranted.

ACKNOWLEDGEMENTS

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1.0 INTRODUCTION

Widespread concerns about the effects of pesticide use on environmental quality and human safety in Alberta during the early 1970's resulted in the initiation of province-wide public hearings by the Environment Conservation Authority (Bones 1974). These hearings provided a comprehensive review of pesticide legislation, registration procedures and application methods in addition to an assessment of the status of pesticide monitoring and research programs within the province. Several years later, the Alberta Fish and Wildlife Division released a position paper outlining the division's concerns and recommendations on the effects of pesticides on wildlife populations (Horstman 1976). While the trend toward increased pesticide use in Alberta was an important concern (Bones 1974), the short and long-term toxicological consequences of continued pesticide use on the natural environment has resulted in an increased awareness by various government agencies and public sector groups (McEwen and Stephenson 1979).

Many of the environmental problems related to pesticide use have centered around the use of DDT (1,1,1,-trichloro-2-2-bis parachlorophenyl ethane) and related chlorinated hydrocarbon insecticides (McEwen and Stephenson 1979). Organochlorine insecticides were extensively used in the 1950's and 1960's, however, because of their environmental toxicity and persistence, use of these compounds has decreased considerably. Instead, use of organophosphate and carbamate insecticides has been increasing steadily (McEwen and Stephenson 1979). Organophosphate and carbamate compounds are known to be acutely toxic to wildlife when orally ingested (Kuhr and Dorough 1976, Stone 1979) but they have a low degree of persistence and exhibit a higher degree of selectivity toward target organisms than the organochlorines (McEwen and Stephenson 1979). Because of these characteristics, use of organophosphate and carbamate compounds for controlling insect pests has increased substantially in Alberta (ECA 1974, Horstman 1976).

As a result of ideal breeding conditions (i.e. warm, dry weather), grasshopper populations in Alberta have increased in recent years. In 1985, Alberta Agriculture issued a forecast that predicted a high potential of grasshopper damage to crop and pasture lands throughout southern Alberta over the next few years (Alberta Agriculture 1985a). In fact, almost 40% of the agricultural

lands from Drumheller south to the U.S. border would require at least some insecticide application. Grasshopper infestations in 1986 were not as severe as those experienced in 1985, however, larger areas of the province were affected (EMR 1986). The decline in grasshopper numbers was primarily a result of the poor breeding conditions observed from late July to early September, 1985 (Johnson and Andrews 1986). While a reduction in grasshopper populations occurred in 1986, moderately warm and dry August weather in 1986 resulted in more egg-laying and will likely result in higher grasshopper populations in 1987 (Johnson and Andrews 1986).

Current spraying programs in Alberta utilize organophosphate and carbamate insecticides for controlling grasshopper damage to grain crops (Alberta Agriculture 1985b). However, because of year-to-year variations in grasshopper populations, the quantity of insecticides applied to control grasshoppers varies widely. For example, in 1976 only 40,000 ha of land were sprayed for grasshoppers (Horstman 1976) while in 1985 and 1986, over 800,000 (Alberta Agriculture unpubl.) and 500,000 (Johnson and Andrews 1986) ha were sprayed, respectively.

Twelve insecticides are currently registered in Alberta for grasshopper control, including carbaryl, carbofuran, decis, dimethoate, malathion, lorsban, hopper stopper, counter 15G, diazinon, monitor, methoxychlor and guthion. Of these, carbofuran (2,3-dimethyl-7-benzofuranyl methylcarbamate) is the most widely used. In 1985, over 52% of the 858,019 ha of treated land was sprayed with carbofuran.

Carbofuran is a broad spectrum systemic insecticide that can be applied in either a liquid or granular form to control grasshoppers and other foliar and soil pests (NRC 1979). While the mechanism of inhibition is not completely understood, it is known that carbofuran toxicity, like other carbamates, is a function of acetylcholinesterase (AChE) inhibition at certain synaptic junctions in the nervous system (Kuhr and Dorough 1976).

Given the amount of insecticide used for grasshopper control in the province, some effects on wildlife may be expected, especially since grasshoppers are considered to be extremely important to wildlife as a nutritious (i.e high protein content), widely distributed and readily available source of food

(McEwen et al 1972). Recent reports of wildlife mortality in southern Alberta have suggested that pesticides may be implicated (Lethbridge Herald, August 7, 1985; Globe and Mail, August 17, 1985). Consequently, a provincial task force comprised of representatives from Alberta Agriculture, Alberta Environment and Alberta Forestry, Lands and Wildlife established a program to examine the environmental effects of grasshopper spraying in Alberta. One of the wildlife components of this program is concerned with insecticide effects on small mammal populations.

To date, the majority of research done on the effect of carbofuran on wildlife has been with birds or have examined the effects of various insecticides on the amount of brain acetylcholinesterase inhibition in mammals under strictly controlled laboratory conditions. While laboratory studies provide important information on lethal dosages (LD_{50}), the response of naturally occuring populations to typical field insecticide applications is not known. Moreover, small mammals serve as an important prey base to many higher order vertebrates (i.e carnivores and raptors) and consequently, if there is any bioaccumulation of carbofuran in small mammals, secondary poisoning of other wildlife species may occur.

Despite the relative importance of small mammal populations to other wildlife species and the extent of pesticide use in Alberta, little research on the short (acute) and long term (subacute) effects of carbamate insecticides on small mammals has been in undertaken. In response to this need, D.A Westworth and Associates Ltd. initiated a preliminary study designed to examine the potential effects of carbofuran spraying on an unconfined population of small mammals in southern Alberta.

1.1 Objectives

The overall objective of the present study was to evaluate the field responses of small mammal populations to carbofuran in southern Alberta. Specific objectives were to:

1. Assess the short-term impacts of carbofuran spraying on field populations of small mammals.

2. Determine the duration of impact and rate of recovery by small

mammal populations following the application of carbofuran.

3. Identify potential impacts of any observed small mammal population changes on raptors and other predators.

2.0 STUDY AREA

The study area is located approximately 3.5 km east of Youngstown, Alberta (Figure 1), occupying an area of about 1.3 sq km within TP 29, R 9, west of the 4th Meridian. Strong and Leggat (1981) place the Youngstown study area in the Short Grass Ecoregion of Alberta. This ecoregion covers approximately 7.1% (47,000 sq km) of the province, making it the largest grassland area in Alberta.

Vegetatively, the Youngstown study area is dominated by herbaceous vegetation. While a large proportion of the area is under cultivation, undisturbed natural areas do occur and are typically vegetated by several grass species. Gramma grass (**Bouteloua gracilis**) with secondary occurrences of spear grass (**Stipa comata**) are characteristic species of the Short Grass Ecoregion (Strong and Leggat 1981). Because of severe moisture deficits that often occur during the summer period, gramma grass is the usual dominant, however, during wetter periods, cover values for spear grass increase considerably. Along seepage areas and moist depressions, scattered aspen and shrub communities of buckbrush (**Symphoricarpos occidentalis**) and wolf willow (**Elaeagnus communtata**) typically occur. By comparison, areas with high water tables (i.e. floodplains and along streambanks), various species of cottonwoods (**Populus** spp.), willows (**Salix** spp.) and water birch (**Betula occidentalis**) dominate the landscape (Strong and Leggat 1981).

The Youngstown study area is drained by Sounding Creek, which flows into Grassy Island Lake. Few permanent waterbodies exist in the immediate vicinity of the study area. Instead, depending on annual moisture conditions, intermittent sloughs are scattered throughout the region (Figure 1). Climatically, the study area is characterized by cold winters, warm summers and low amounts of precipitation. This continental prairie climate results in summer and winter precipitation values that are among the lowest in the province. Meteorological information from the Oyen weather station indicates an annual daily temperature of $2.6^{\circ}C$, however, mean daily temperatures range from $-15.8^{\circ}C$ in January to $17.6^{\circ}C$ in July (Environment Canada 1982). Annual winter and summer precipitation values are 107.8 mm and 245.6 mm, respectively. The growing season in the vicinity of Youngstown only varies from 100 to 125 days (Strong and Leggat 1981).

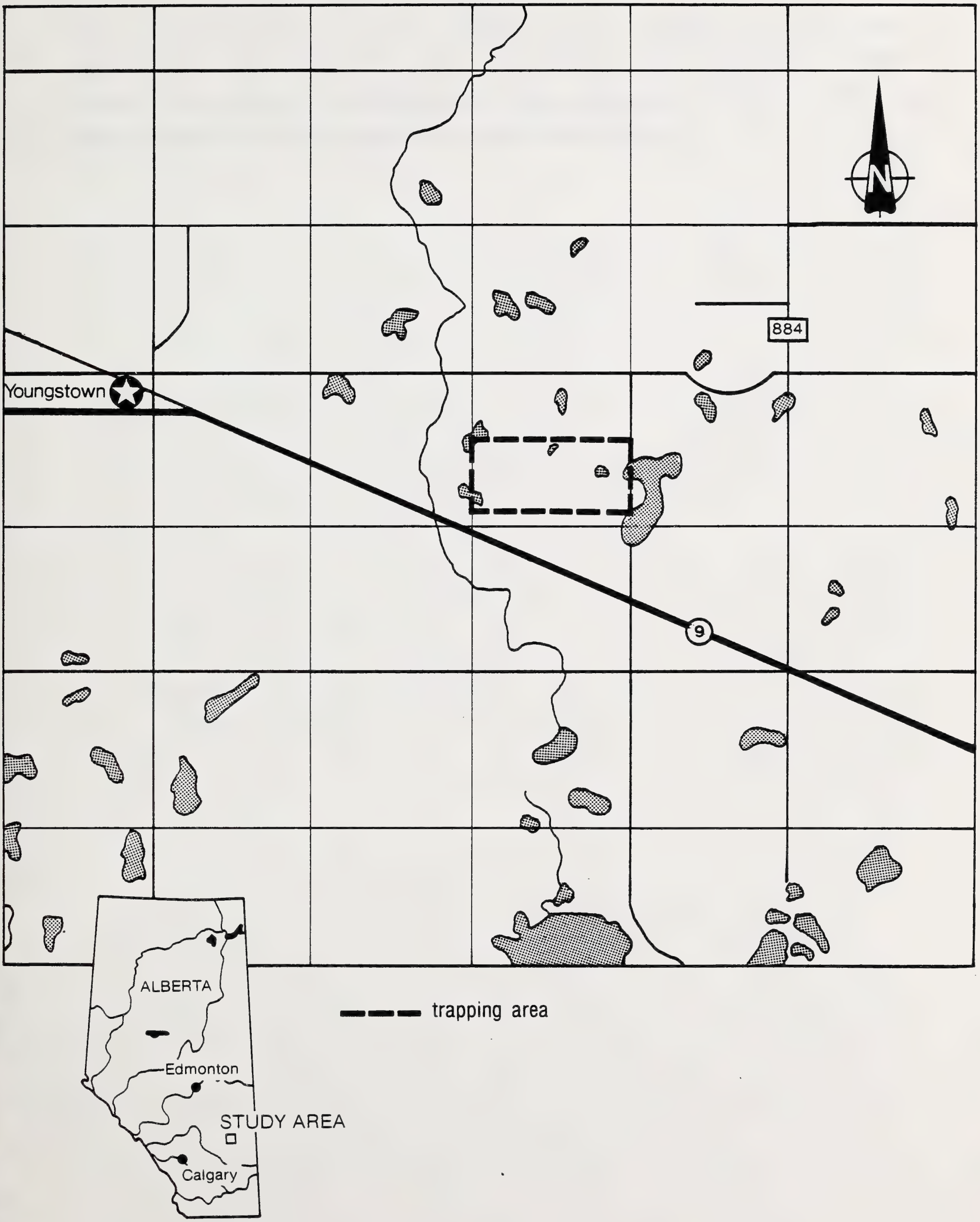

Figure 1. Location of the Youngstown study area, 1986.

The principal land use in the vicinity of the Youngstown study area is agriculture. Crop production on both irrigated and unirrigated lands is primarily comprised of growing wheat, oats and barley. Native and disturbed pasture lands are often utilized for cattle production.

3.0 METHODS

3.1 Site Selection and Study Design

Based on an examination of provincial grasshopper forecast maps prepared by Alberta Agriculture, a general study area in the vicinity of Youngstown, Alberta was selected. A number of criteria were considered during the selection process including extent and severity of the grasshopper problem, presence of representative habitats, general use of the insecticide carbofuran and the presence of landowners interested in participating in the study. The Youngstown area was considered suitable since it was in an area that was rated as having moderate infestations of grasshoppers over the last two years (Johnson and Andrews 1986) and landowners have made considerable use of carbofuran to control grasshopper populations in the past (G. Andrews, pers. comm.). In 1985, approximately 55% of the area in Region II was sprayed with carbofuran (Alberta Agriculture, unpubl.).

Following a review of county maps and 1:60,000 black and white aerial photography, four sample sites were selected in an area located approximately 3.5 km east of Youngstown. Sites 1 and 2 were established on disturbed unmowed pasture land (Photo 1) while Sites 3 and 4 were placed in wetland (slough) habitats (Photo 2). One sample site from each habitat type was considered as a control while the second site from each habitat was treated as experimental. Both experimental and control sites within each habitat type were comparable with respect to moisture regime, aspect and slope. In addition, control sites were located far enough away that they would not be exposed to spray drift once the insecticide was applied to the experimental sites.

Arrangements were made with a local landowner to undertake a spraying program on the experimental sites. Spraying for grasshopper control in the Youngstown area normally occurs from mid-June to mid-July. Efforts were made to coordinate spraying activities with the landowner, however, because of weather conditions and the availability of spraying equipment, the experimental sites were not sprayed with carbofuran until 2 July, 1987. At this time, Sites 1 and 3 were sprayed with carbofuran at an application rate of 275 ml/ha (Alberta Agriculture 1985b) using a truck-mounted 6 m boom

Photo 1. Prairie habitat types sampled in the Youngstown study area are comprised of disturbed unmowed pasture lands that typically support low density populations of small mammals.

Photo 2. Slough habitats in the Youngstown study area are characterized by dry wetland beds that are intermittently flooded throughout the summer. Small mammal densities in these habitats are considerably higher than those in prairie habitat types.

mist sprayer (Photo 3).

Carbofuran is a relatively new insecticide developed by Chemargo in 1967 and marketed under the registered trade name Furadan. Carbofuran is usually applied in granular form or as a flowable, with a soil half-life of 30 to 80 days (Kuhr and Dorough 1976). When used according to label directions, it is non-phytotoxic, however, the mammalian toxicology of carbofuran is unusual in that the oral LD_{50} is very low for the rat (11 mg/kg), yet the dermal LD_{50} is in excess of 10,000 mg/kg (Hudson et al. 1984). Mice are particularly sensitive to carbofuran since the reported LD_{50} is 2 mg/kg of body weight (NRC 1979, Drabek and Neumann 1985). The LD_{50} value reflects the dosage of an insecticide required to kill 50% of an experimental population.

3.2 Livetrapping Methodology

Livetrap-tag-recapture procedures were used to estimate population densities. Depending on the size of each habitat unit, livetrapping grids ranged in size from 42 trapping stations at Site 4 to 120 stations at Site 2. Grids were established using a compass and forestry hip chain. Within each grid, trapping stations were placed at 20 m intervals and marked with galvanized wire stakes and orange flagging. Two Sherman livetraps were placed along runways or other suitable locations within a 2m radius of each station. In the case of sites 3 and 4, trapping stations were established at likely locations (areas of animal activity or suitable cover) around the margins of dry slough beds. To minimize the effects of thermal stress, the traps were provided with fortrel bedding material and covered with such natural materials as grasses and leaves or in the case of more exposed locations, a 15 x 30 cm plywood cover was used. All traps were baited with a mixture of oats and peanut butter.

Each grid was livetrapped for six consecutive days during the pre-spray trapping session conducted between 18 June and 25 June, 1987. Because of frequent rains and the unavailability of spraying equipment from 26 June to 1 July, carbofuran was not applied to the experimental plots until 2 July, 1987. Immediately after the spraying program, the livetraps were reset and each grid was trapped for three consecutive days. This period was

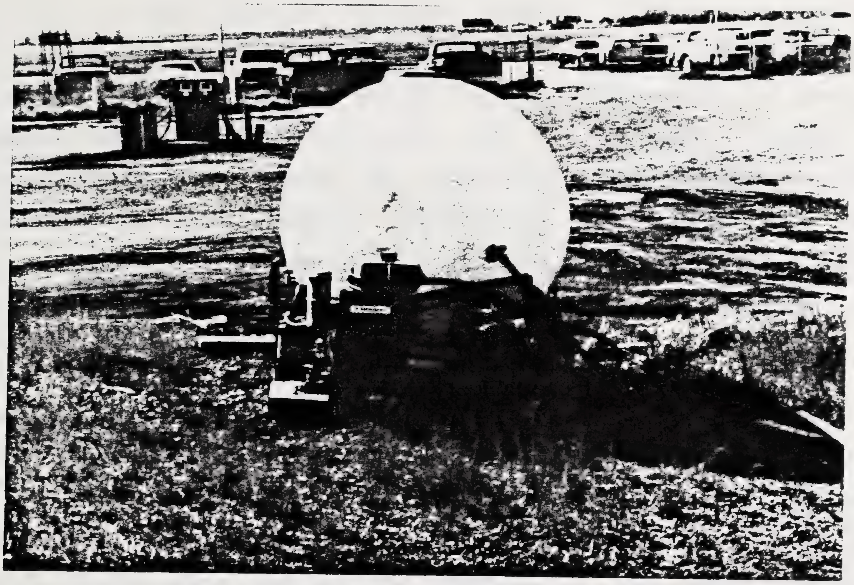

Photo 3. A truck-mounted mist sprayer was used to spray the experimental sites within each habitat type prior to the post-spray trapping session.

Photo 4. Livetrapping techniques were used to obtain population estimates of small mammals on the control and experimental sites of each habitat type. Captured animals were tagged, weighed and released.

considered as the post-spray trapping session. A late summer trapping session was conducted from 15 August - 19 August, 1987. The data generated during the pre-spray trapping session provided information on pre-exposure population levels of small mammals while the post-spray trapping session provided a basis for assessing the short-term (acute) effects of carbofuran applications on small mammal populations. Information from the late summer trapping session was used to compare the reproductive condition of tagged animals to earlier trapping sessions and to derive survival estimates of tagged animals.

During each trapping session, traps were checked twice daily: once in the morning and evening. Animals captured for the first time were marked with an aluminum ear tag, weighed (g) and sexed (Photo 4). Reproductive condition was determined by the position of the testes (inguinal, abdominal, scrotal) in the male and by the condition of the vagina (perforate or imperforate), pubic symphysis (open or closed) and nipple condition (visible, not visible or lactating) in the female. This information along with the time and date of capture was recorded on coding sheets for later data analysis.

3.3 Tissue Collection and Analysis

Small mammals were collected from control and experimental sites between 16 and 20 hrs following the spraying program on 2 July, 1987. Montz and Kirkpatrick (1985a) found that brain AChE activity of **Peromyscus leucopus** was relatively unaffected within 6 hrs of dosing with either diazinon (Phosphorothioic acid, O,O-diethyl O- 6-methyl-2-(1-methylethyl)-4-pyrimidinyl ester) or parathion (Phosphorothioic acid, O,O-diethyl O-(4-nitrophenyl) ester), two organophosphate insecticides. Following this latent phase, AChE activity declined rapidly 6-12 hrs after dosing. Maximal AChE inhibition occurred between 12 and 24 hrs after which levels increased to those recorded for control animals (Montz and Kirkpatrick 1985a).

All small mammals found in the live-traps were placed in plastic bags and sacrificed by CO_2 asphyxiation. Gastro-intestinal tract and brain tissues were removed from each animal, placed in labelled glass vials and immediately frozen in liquid nitrogen for later laboratory analyses. AChE activity was colometrically determined following the method described by

Ellman et al. (1961) while carbofuran residue analyses were confirmed and quantified by single ion monitoring on mass-spectrometry. AChE activity determinations and residue analyses were coordinated and administered by the Alberta Fish and Wildlife Division and conducted by the Alberta Environmental Centre in Vegreville, Alberta and the Food Laboratory Services Branch of Alberta Agriculture in Edmonton, Alberta, respectively.

3.4 Data Analysis

Population estimates were derived using two methods. The first, which is commonly referred to as the Jolly-Seber method (Overton and Davis 1969) is based on the ratio of marked to unmarked animals in a population during each trapping session. The Jolly-Seber estimator takes into account immigration, emigration and death of animals during each trapping session. The second method is based on the minimum number of animals known to be alive in each sampling period. Both techniques tend to be complementary since the minimum method, although it may underestimate population densities by 10 to 20% (Hillborn et al. 1976), will provide an estimate on occasions when the number of recaptures is not sufficient for calculating a Jolly-Seber estimate. Survival rates between each trapping session were simply calculated on the basis of the following ratio: number of tagged animals known to be alive at time t+1 divided by the number of originally tagged animals known to be alive at time t (Krebs 1972).

Chi-square contingency table analysis was used to compare the reproductive condition (reproductive versus non-reproductive) of females and males by trapping session. Because of small samples sizes, only scrotal males and perforate or pregnant females were classed as being in reproductive condition. The remaining animals were considered as non-reproductive. The null hypothesis that there is no difference in reproductive condition of females or males between control and experimental sites during each of the three trapping sessions was tested at a probability level of $P < 0.05$.

Differences in brain AChE activity between control and experimental plots and between sex were tested using analysis of variance (ANOVA) procedures at a probability level of $P < 0.05$. Because of unequal variances and non-normal distributions, the AChE data set was logarithmically (LG10)

transformed. Logarithmic transformations are particularly appropriate when the means are positively correlated with their variances (Kleinbaum and Kupper 1978, Sokal and Rohlf 1981). Multiple classification analysis (MCA) was used to examine the specific levels of each significant factor resulting from ANOVA.

All analyses were performed using the various procedures available with the SPSS/PC+ (Norusis 1986a) and SPSS/PC+ Advanced Statistics (Norusis 1986b) program packages and were executed on an IBM PC/XT microcomputer.

4.0 RESULTS

4.1 Small Mammal Demography

4.1.1 Population Estimates

During the 1986 livetrapping program, 355 cricetid rodents were tagged including 283 **Peromyscus maniculatus,** 68 **Microtus pennsylvanicus** and 4 **Mus musculus.** In addition, 34 **Spermophilus tridecemlineatus** were captured and tagged. Since **P. maniculatus** comprised approximately 80% of the tagged small mammal population, only this species was used for detailed analyses of the effects of carbofuran applications. Overall, 16 days of trapping effort on 4 sample sites yielded 1112 captures of the 389 small mammals. Trap mortality during the trapping sessions averaged 4.1%, with the highest mortality recorded for **M. pennsylvanicus** (6.5)%.

Numbers of **P. maniculatus** differed considerably between the two habitat types sampled and between experimental and control sites within each habitat type, however, populations were remarkably similar among the three trapping sessions (Figure 2). **P. maniculatus** populations in slough habitats were approximately three times higher than those observed on prairie types and were approximately 33% higher on experimental sites of each habitat type than on the control sites.

With the exception of the pre-spray trapping session, numbers of **M. pennsylvanicus** were generally similar across all sites and habitat types (Figure 3). A dramatic decline in **M. pennsylvanicus** numbers from the pre-spray levels was observed following the carbofuran application (post-spray trapping session) at Site 1. While a decrease in **M. pennsylvanicus** numbers during the post-spray trapping session at Site 3 was also noted, it was not considered significant (Figure 3). **M. pennsylvanicus** populations at three of the sites (1, 2 and 3) were also at least 50% lower in the late summer trapping session than during the post-spray session.

4.1.2 Survival Rates

Survival rates were considered as the proportion of marked animals not lost through death or emigration. Although the term survival is used for

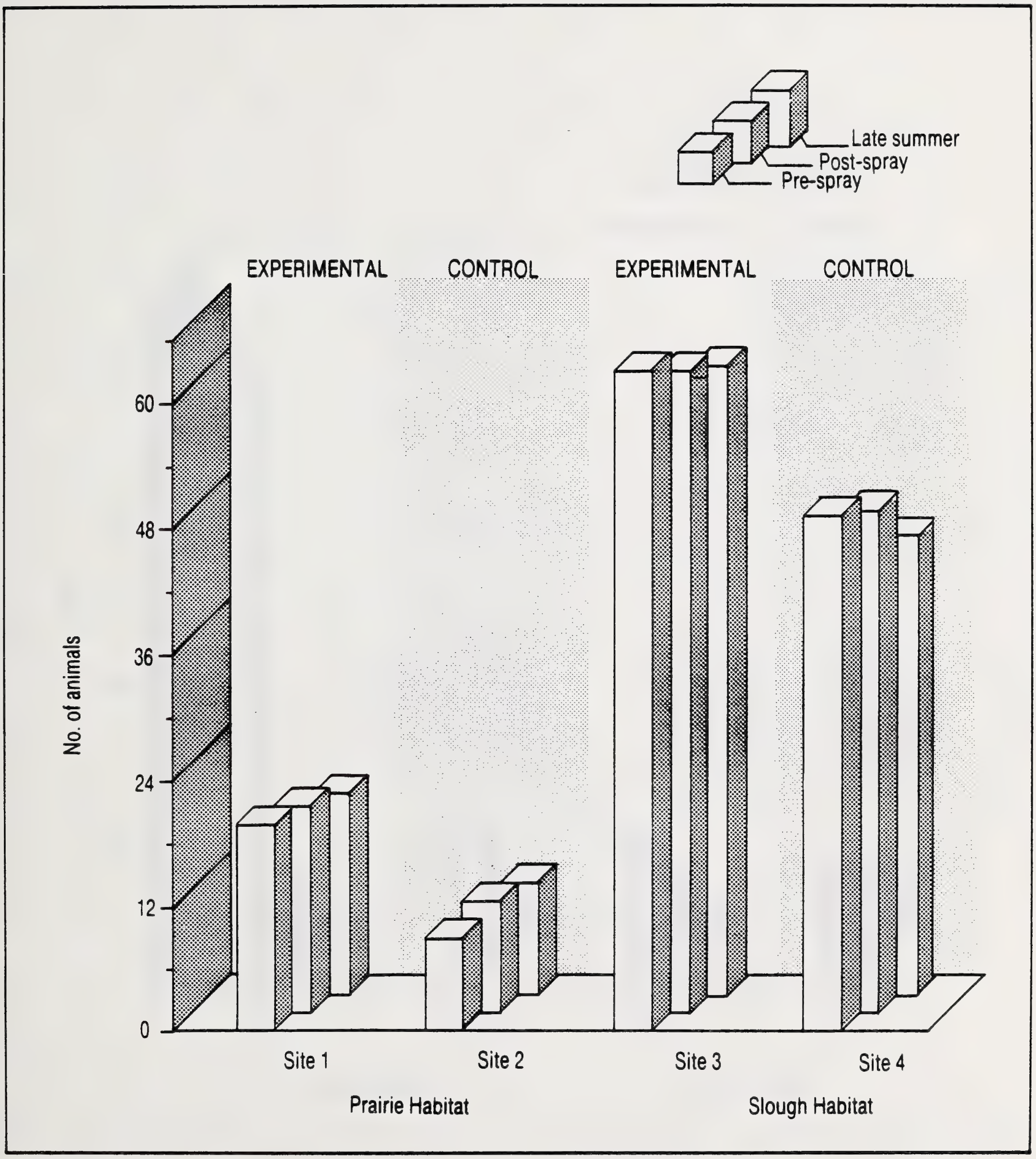

Figure 2. Population estimates of *Peromyscus maniculatus* by trapping session, site, treatment and habitat type in the Youngstown study area, 1986.

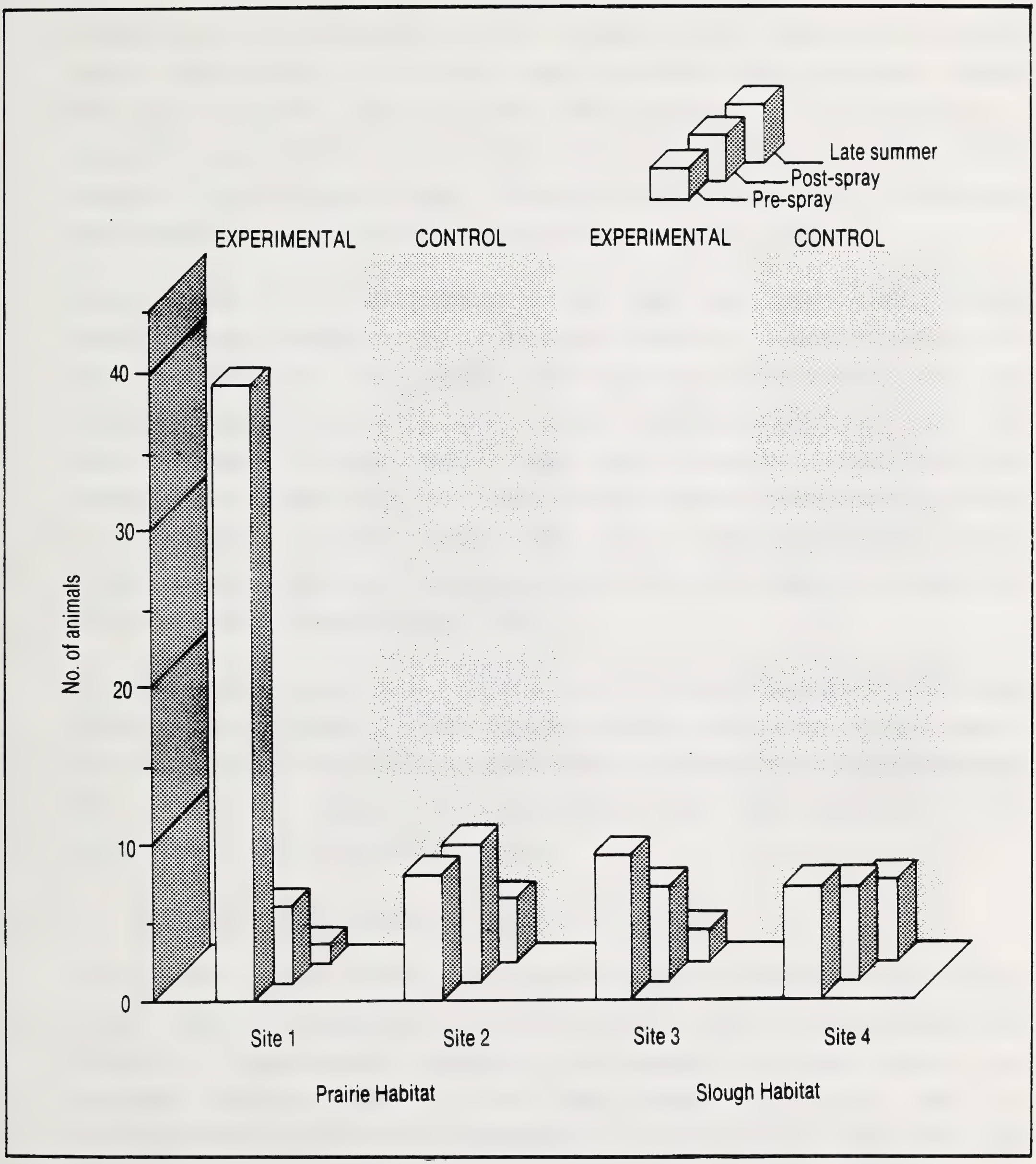

Figure 3. Population estimates of *Microtus pennsylvannicus* by trapping session, site, treatment and habitat type in the Youngstown study area, 1986.

consistency with the literature, it is a measure of the proportion of marked animals remaining on a site and is more correctly termed residency (Mihok 1979). In the present study, survival was calculated as the proportion of originally tagged animals that survived from time t to time t+1 (Krebs 1972). Because of small sample sizes, estimates were calculated on a treatment basis (control or experimental site) regardless of habitat type.

Survival rates for **P. maniculatus** in the young age cohort were similar among trapping sessions on the control sites (Table 1). However, survival of the adult portion of the population was low (0.400) between the pre- and post-spray trapping sessions. Adult survival increased substantially over the summer period. A similar trend in adult survival was also observed on the experimentally sprayed sites but low survival rates were recorded for young **P. maniculatus.** Overall survival rates for **P. maniculatus** were similar among trapping sessions in the control sites but were considerably lower on the experimentally sprayed sites (Table 1).

Because of small sample sizes, survival rates for **M. pennsylvanicus** were only calculated on the basis of total animals present rather than by age cohort. The results indicate that while overall rates of survival for **M. pennsylvanicus** were low on the control sites, even lower rates were observed on the experimentally sprayed sites (Table 1).

4.1.3 Reproductive Condition and Weight Comparisons

The majority of adult males and females captured throughout the trapping sessions were in reproductive condition (Table 2). The only significant difference in reproductive condition was detected for males during the post-spray trapping session. In this case, higher numbers of males in non-reproductive condition were observed on the experimental sites than was expected while the opposite was true for reproductive males (Table 2).

The overall sex ratio (proportion of males) for **P. maniculatus** throughout the trapping session was calculated as 0.49, however sex ratios varied considerably between control and experimental sites as well as among trapping sessions (Table 2). The proportion of males observed on experimental sites was considerably higher during the post-spray trapping session (0.62) than what was recorded during the late summer session (0.34). By

Table 1. Survival rates of **Peromyscus maniculatus** and **Microtus pennsylvanicus** by treatment, age cohort and trapping session in the Youngstown study area, 1986.

Treatment	**Age Cohort**	**N**[1]	**Survival Rate**	
			Pre- to Post-spray	**Post-spray to Late Summer**
			Peromyscus maniculatus	
Control	Adult	10	0.400	0.750
	Young	33	0.667	0.682
	TOTAL	43	0.605	0.692
Experimental	Adult	16	0.438	0.857
	Young	47	0.574	0.296
	TOTAL	63	0.540	0.412
			Microtus pennsylvanicus	
Control	TOTAL	15	0.333	0.666
Experimental	TOTAL	30	0.200	0.333

[1] Indicates the number of tagged animals used for survival rate calculations.

Table 2. Numbers of **Peromyscus maniculatus** in reproductive condition by sex, treatment and trapping session in the Youngstown study area, 1986.

Sex	Condition	Pre-spray Session			Post-spray Session			Late Summer Session		
		Control	Sprayed	χ^2	Control	Sprayed	χ^2	Control	Sprayed	χ^2
Female	Non-reproductive	14	18	0.265	6	5	0.500	3	22	0.240
	Reproductive	7	17		4	5		6	18	
Males	Non-reproductive	9	15	0.292	3	14	0.005	9	16	0.655
	Reproductive	18	17		6	2		4	5	
Total	Non-reproductive	23	34	0.825	9	19	0.079	15	49	0.001
	Reproductive	25	34		10	7		36	33	

comparison, male sex ratios on control sites decreased slightly during the post-spray session and were back to pre-spray levels during the late summer trapping session (Table 2).

Overall, these results indicate a lack of any general pattern of carbofuran inhibition on the reproductive condition of **P. maniculatus.** As was the case with reproductive condition, there were no obvious weight differences observed for **P. maniculatus** between control and experimental sites and among trapping sessions that could be attributed to carbofuran exposure (Table 3). A 3 g weight loss was noted for non-reproductive males during the post-spray trapping session but only a marginal difference in weights existed between the post-spray and late summer trapping sessions.

4.2 Carbofuran Toxicity

The presence of any insecticide in the tissues of wildlife is likely related to food intake (i.e ingestion) and presumably reflects the extent of contamination of the food supply. To assess this possibility, brain and gastro-intestinal tissues were collected following the application of carbofuran. Thirty-four small mammals were collected for tissue analyses, of which only two were **M. pennsylvanicus.** Consequently, brain acetylcholinesterase activities and insecticidal residues were only determined for **P. maniculatus.**

4.2.1 Brain Acetylcholinesterase Activity

Brain AChE activity was examined on the basis of two parameters: (1) the number of units per mg of protein and (2) the number of units per g of tissue where one unit of AChE activity is equal to 1 uMol of acetylcholine hydrolyzed per min. No significant differences ($P > 0.05$) in mean brain AChE activities of **P. maniculatus** were detected for either treatment or sex (Table 4). The mean number of units of AChE activity for both protein and tissue were virtually identical in the control and experimental sites (Table 5), suggesting that carbofuran had no inhibitory effects on brain AChE levels.

4.2.2 Tissue Residues

Since larger amounts of tissue are typically required for these analyses, the

Table 3. Mean weight (gm) comparisons of non-reproductive animals and reproductive males of **Peromyscus maniculatus** by treatment and trapping session in the Youngstown study area, 1986.

Treatment	**Trapping Session**								
	Pre-Spray			**Post-Spray**			**Late Summer**		
	$\bar{x}$	**S.D.**	**N**	$\bar{x}$	**S.D.**	**N**	$\bar{x}$	**S.D.**	**N**
				Non-reproductive Animals					
Control	22.0	3.6	8	23.3	7.5	4	19.8	3.4	4
Experimental	22.2	3.1	6	19.4	3.6	9	19.8	3.4	4
				Reproductive Males					
Control	20.1	2.5	7	19.4	3.4	5	20.3	2.4	15
Experimental	20.0	-	1	20.0	1.4	2	21.7	2.1	7

Table 4 . Analysis of variance of transformed* acetycholinesterase activity measurements by treatment and sex for **Peromyscus maniculatus** brain tissues in the Youngstown study area, 1986.

Source of Variation	Degrees of Freedom	Units/mg Protein			Units/g Tissue		
		MS**	F	Sign.	MS	F	Sign.
Main Effects	2	0.000	0.128	0.880	0.000	0.098	0.907
Treatment	1	0.001	0.238	0.630	0.000	0.035	0.853
Sex	1	0.000	0.000	0.986	0.001	0.189	0.667
2-Way Interactions	1	0.013	5.718	0.024	0.016	5.300	0.30
Treatment by Sex	1	0.013	5.718	0.024	0.016	5.300	0.30
Explained	3	0.005	1.992	0.140	0.006	1.832	0.166
Residual	26	0.002			0.003		
TOTAL	29	0.003			0.003		

*Lg 10(x)

**Mean Square

Table 5. Brain acetylcholinesterase activity of **Peromyscus maniculatus** by treatment and sex in the Youngstown study area, 1986.

Treatment	Sex	N[1]	Units/mg Protein		Units/g Tissue	
			x	S.D.	x	S.D.
Control	Both	10	0.1011	0.0105	9.6950	1.0985
	Female	6	0.0955	0.0094	9.0250	0.8794
	Male	4	0.1095	0.0053	10.7000	0.2944
Experimental	Both	20	0.1036	0.0128	9.6890	1.3895
	Female	7	0.1086	0.0137	10.0886	1.6109
	Male	13	0.1009	0.0119	9.4738	1.2714

[1] One unit of acetylcholinesterase activity = uMol of acetylcholine hydrolyzed/minute.

gastro-intestinal tracts of mice collected from control and experimentally sprayed sites were seperately composited. Ten gastro-intestinal tracts were composited to form the control sample while 20 tracts were used for the sprayed sample. Residue analysis was used to determine the presence of the parent compound, carbofuran and one of its metabolites, 3-hydroxy carbofuran in the gastro-intestinal tracts.

In contrast to brain AChE activity, carbofuran residues (2.0 ppm) were detected in the gastro-intestinal tracts of **P. maniculatus** collected from the experimentally sprayed sites. By comparison, no carbofuran residues were observed for mice taken from the control sites, based on a minimum dectectable level of 0.25 ppm. The carbofuran derivative, 3-hydroxy carbofuran was not found in either the control or experimental sites.

5.0 DISCUSSION

In interpreting the results of the present study, several points should be considered. The present study was designed to provide a preliminary assessment of the effect, if any, on unconfined small mammal populations of carbofuran exposure at the field application rates currently used in southern Alberta. In addition, **a priori** knowledge of small mammal densities in the Youngstown area did not exist and although the resulting small mammal population sizes were considered small, the study design ensured that valid comparisons could be made between control and experimental sites. Morris (1970) stated that some care must be taken in interpreting the population results following insecticidal treatment since population responses may be masked or confused by normal population processes. In the present study, habitat homogeneity, trapping grid size, and the proximity of control and experimental sites within each habitat type were important considerations prior to the initiation of field sampling. Therefore, if the experimental sites did exhibit significant deviations in any of the variables examined from the control areas, this should reflect the influence of carbofuran on small mammal populations.

5.1 Population Dynamics

P. maniculatus was clearly the most abundant cricetid rodent in the study area, followed by **M. pennsylvanica** and **Mus musculus.** While large habitat-related differences existed, there were no readily apparent effects on absolute numbers of **P. maniculatus** following carbofuran exposure on the experimental sites in either habitat type. In fact, population levels on control and experimental sites in both prairie and slough habitats were remarkably similar. In contrast, **M. pennsylvanicus** populations were similar in both habitat types but declined following carbofuran treatment.

Initially, it would seem that exposure to carbofuran influenced **M. pennsylvanicus** and did not affect **P. maniculatus** populations. However, low survival rates observed for tagged animals of both species on the experimental sites during the post-spray and late summer trapping sessions suggests there were some direct toxic effects. Because overall survival rates on control sites were higher than those recorded on the experimental grids

and rates did not vary significantly among the three trapping sessions, the low survival rates observed on the experimental sites appear to reflect a carbofuran-induced population response.

Since population levels for **P. maniculatus** remained relatively stable and survival rates were low throughout the three trapping sessions, tagged animals that could not be accounted for following the pre-spray trapping session must have either left the experimental trapping grids or suffered carbofuran related mortality. The latter situation is more likely because significant amounts of carbofuran residues were detected in the gastro-intestinal tracts of mice collected from the experimental sites. The amount of carbofuran residues found in mice in the present study is identical to the LD_{50} value of 2.0 ppm reported for laboratory mice (NRC 1979, Drabek and Neumann 1985), a dosage value that would result in a 50% mortality rate of a laboratory population. Therefore, the low observed survival rate for both cricetid rodent populations and the significant amount of residues present strongly suggests that some mortality must have occurred even though no dead or dying mice were found on the experimental trapping grids. This was expected since animals suffering from carbofuran intoxication would likely return to their burrows or nests, making the location of dead animals virtually impossible. In addition, the observed stability of the **P. maniculatus** population among the three trapping sessions suggests that there may have been some immigration from adjacent habitats, natality rates had increased or a combination of both. In either case, young animals comprised approximately 90% of the newly tagged animals during the post-spray and late summer trapping sessions.

The significance of either carbofuran-induced movements (emigration or immigration) and/or mortality to small mammal populations in the Youngstown study area is difficult to ascertain at this time since the present study was primarily designed to investigate the overall presence or absence of an insecticide effect. While other studies have shown that small mammal populations respond to various insecticides in different ways, comparisons are often difficult to make not only among major groups of insecticides but also within groups. This is especially true for carbofuran since no other research has been undertaken to date that has evaluated the effects of this insecticide on field populations of small mammals. Within the carbamate

group, Pillmore et al. (1971) evaluated the laboratory and field responses of birds and small mammals to Zectran (4-dimethylamino-3,5-xylyl methylcarbamate) exposure over a three year period, a carbamate insecticide used for spruce budworm control in northwestern United States. While the acute dosage studies conducted in the laboratory indicated that birds were more susceptible to Zectran than small mammals, no detrimental effects on on either group could be demonstrated using the recommended field application rates. Similar results were reported by Sarrazin and McLeod (1975) for the carbamates Zectran and Matacil (4-dimethylamino 3-methylphenyl N-methylcarbamate) used for spruce budworm control in eastern Canada.

Carbaryl (1-naphthyl N-methylcarbamate) is another widely used carbamate insecticide for which some small mammal studies have been conducted. Connor (1960) found that a single application of carbaryl at the prescribed field rate was not harmful to terrestrial wildlife (birds and small mammals) however the long-term effects were not examined. In contrast, Barrett (1968) found that carbaryl treatment resulted in a reduction in density, biomass and diversity of primary and secondary consumers in a study of acute, low level insecticidal stress on a grassland ecosystem. While **P. polinotus** seemed to be unaffected by carbaryl treatments, **Sigmodon hispidus** populations appeared to be measurably influenced by insecticide application. In a follow-up study, Pomeroy and Barrett (1975) assessed the effects of carbaryl applications on **P. polinotus, Mus musculus** and **S. hispidus** populations over a 1-year period. In this study, they concluded that even though the resources available to populations of small mammals were similar in the control and insecticide treated areas, population composition for each of the species were significantly altered for several months following carbaryl treatment.

Conflicting results seem to characterize studies designed to evaluate the field effects of organophosphate insecticides on small mammal populations as well. Barrett and Darnell (1967) undertook an intensive study of small mammal populations in an Indiana clover field before and after spraying with dimethoate (0,0-dimethyl-s- n-methylcarbomoylmethyl -phosphorodithioate). Numbers of **P. maniculatus** did not change appreciably following the spraying program, however, numbers of **M. ochrogaster** increased and **Mus musculus**

populations decreased. They concluded that **P. maniculatus** were seemingly unaffected by the insecticide or by changes in interspecific competition, but dimethoate-induced changes in the food chain accounted for the observed differences in **M. ochrogaster** and **Mus musculus** populations. Since **Mus musculus** is considered an omnivore, reduction in the insect population likely caused animals to leave the treated area but this was offset by a concomitant increase in **M. ochrogaster** numbers, a species considered to have herbivorous food habits. By comparison, Buckner and McLeod (1975) and Buckner et al. (1975) reported no evidence of insecticide impact on small mammal populations of fenitrothion and Orthene spraying on eastern Canadian forests.

The results obtained in the present study are similar to those reported by Jett et al. (1985) in their quantitative study of the effects of ground applications of Orthene on unconfined populations of **M. pennsylvanicus** in Maryland, U.S.A. During the first year of study, no evidence was found to suggest that population size, survival probability or recruitment of **M. pennsylvanicus** were significantly affected after Orthene treatments. Population sizes between the control and treated plots in the second year of study were also similar but survival and recruitment rates were lower than expected suggesting that some treatment effects were present. Jett et al. (1985) felt that the animals had either left the treated trapping grids or were directly or indirectly killed by the insecticide. The resulting 'biological vacuum' (Stickel 1946, Calhoun and Webb 1953) appeared to be rapidly filled from adjacent populations because the number of **M. pennsylvanicus** in both the treated and control grids remained relatively stable and a high post-spray recruitment rate was observed on the treated trapping grids (Jett et al. 1985).

More dramatic population responses were reported by Morris (1970, 1972) in his long-term study of the effects of endrin exposure on **P. maniculatus** and **M. pennsylvanicus** populations. Endrin is a persistent, highly toxic organochlorine pesticide. He not only observed significant reductions in **M. pennsylvanicus** populations immediately following the application of endrin, but also disruptions in the social structure of a previously stable population. The original stable population appeared to be replaced with an unstable population comprised of a combination of the original residents and a large

proportion of transient immigrating animals. Morris (1970) felt that this unstable situation would continue for some time afterward until normal population regulatory processes become activated to keep numbers at the habitat carrying capacity. He also observed differential species responses to endrin treatment. The **P. maniculatus** population was more severely affected than the **M. pennsylvanicus** population, suggesting a more complete toxic response and a long-term effect exists in addition to the initial post-spray reduction in numbers of **P. maniculatus.**

5.2 Reproductive Performance

Small mammals generally rely on high reproductive rates to maintain population levels and consequently, any reduction in reproductive capabilities may more adversely affect a population over the long-term than the loss of some animals through direct carbofuran mortality. Therefore, various reproductive characteristics of small mammals were evaluated during the field sessions in an effort to determine the subacute effects of carbofuran exposure on reproductive performance. Since sample sizes were small, all of the indicators could not be assessed. However, based on mean weight and breeding condition comparisons between the control and experimental sites and among the three trapping sessions, reproductive performance of **P. maniculatus** in the Youngstown study area did not appear to be significantly affected. There were, however fewer reproductive males on the experimental sites than expected, suggesting sexes may exhibit differential responses to carbofuran exposure.

Other field studies have reported variable reproduction responses to different insecticides on the basis of sex, reproductive condition and litter sizes. Jett et al. (1985) found that of the reproductive parameters studied (lactation, vaginal and pubic symphysis condition) over a two year period, no significant effect from Orthene treatment could be demonstrated. They did, however, note a decline in the number of pregnant females on the treatment grid following spraying during the second year of study. In contrast, carbaryl has been shown to inhibit or delay reproduction, sometimes leading to significant alterations in the population dynamics of small mammal communities (Connor 1960, Barrett 1969, Pomeroy and Barrett 1975). The herbicide Roundup, did not affect reproduction of **P. maniculatus** populations in British Columbia one

year after treatment (Sullivan and Sullivan 1981). Similarly, Morris (1968, 1970, 1972) found that endrin, an organochlorine, did not significantly affect reproductive performance of surviving animals. Field applications of Zectran did not have any observable effects on forest-dwelling small mammal and bird populations (Pillmore et al. 1971), however, in laboratory studies, the reproductive capacity of surviving animals was found to severely affected (Tucker and Crabtree 1969).

Laboratory studies generally indicate a more toxic effect on reproductive parameters of various animal species. These toxic effects are primarily a result of oral applications at higher dosages than would be normally encountered in field situations. Nevertheless, these studies demonstrate the possible subacute effects of insecticidal exposure on animal reproduction. In a three generation laboratory study of albino rats, 50% of the females that were fed up to 100 mg/kg of body weight of carbofuran lost their entire litters 21 days post-partum, even though mating and relative fertility rates were similar to control groups (Kennedy et al. 1967; cited in NRC 1979). Similarly, Arnold et al. (1967; cited in NRC 1979) reported that male rats fed up to 30 mg/kg of body weight of carbofuran exhibited reductions in general body weight and in weights of such organs as the liver, brain, kidney and adrenal glands. However, these effects were not observed in treated females, suggesting that sex differences may account for some of the variable susceptibility to carbofuran. In contrast, Kennedy et al. (1967; cited in NRC 1979) did not find any sex related differences while Dorough and Kuhr (1976) suggest that females are generally more susceptible to carbamate intoxication. Montz and Kirkpatrick (1985b) reported that the additional demands of growth, pregnancy and lactation may predispose adults to less insecticide intolerance. But in a study of the organophosphate parathion on **Sylvilagus floridanus**, two dosages of 8 mg/kg of body weight orally applied 30 days apart did not significantly influence litter size, however, food intake was reduced for two days (Montz et al. 1984). Six to 10 days after the parathion treatment, compensatory feeding had occurred. Montz et al. (1984) concluded that the subsequent increases in ingestion functioned in offsetting the initial depressant effect on food intake. Linder and Richmond (1985) examined the subacute effects of carbofuran granules on **M. ochrogaster**. They found that while the granules were acutely toxic,

they appeared to exert only minimal effects on the reproductive performance of the animals.

Based on extensive use of laboratory mice and rats, the lethal and sublethal effects of carbofuran (enzyme production, reproduction, general activity levels, tumor production and teratogenic effects) are well known (McEwen and Stephenson 1979). However, most of these effects are generated under higher dosages than would normally be used during field applications and the consequences of widespread chronic use of carbofuran and many other carbamate and organophosphate insecticides in Alberta is virtually unknown.

5.3 Mechanisms of Carbofuran Toxicity

Insecticides may influence wildlife populations in a number of ways and in the context of the present study, five possible scenarios exist. The first and most obvious scenario is one of no effect. In this case, the measured population parameters on the experimental plots are not appreciably different from the control sites following carbofuran treatment. Secondly, if an effect is determined, are the field application rates of carbofuran having any direct toxic (acute) effects (i.e. mortality) on small mammal population dynamics and, if so, what is the extent of carbofuran toxicity. Third, are there any subacute effects (i.e. reduced reproductive performance) that may influence small mammal populations exposed to field applications of carbofuran. Fourth, are animals exposed to carbofuran showing any indirect toxic effects (i.e. changes in interspecific and intraspecific behaviors). A fifth scenario and perhaps most important, is concerned with the potential long-term effects of repeated carbofuran exposure on the viability of small mammal populations and ultimately, on higher-order vertebrates that depend on small mammals as prey.

Even though carbamate insecticides are highly toxic to wildlife when ingested, they have become increasingly important in controlling agricultural pests because of their low environmental persistence and selective toxicity (Kuhr and Dorough 1976). In efforts to monitor the potential effects of these insecticides, brain AChE levels are often used as an indicator of intoxication for many wildlife species. Like organophosphates, carbamate insecticides function by affecting nerve impulse transmission at the synapse. For

example, peripheral cholinergic synapses control a number of vital body functions including heartbeat and respiration. These synapses are stimulated by acetylcholine (ACh) and are 'shut off' by AChE. Carbamate insecticides act by interfering with cholinergic transmission and inhibiting AChE so that it cannot cleave ACh (Dorough and Kuhr 1976), resulting in prolonged stimulation and eventual nerve failure. While the symptomology of organophosphate and carbamate intoxication in mammals is similar, a critical difference in their modes of action exists. Carbamate insecticides are considered reversible AChE inhibitors while organophosphate inhibition is considered to be less reversible (Dorough and Kuhr 1976, NRC 1979).

The significance of carbofuran residues in small mammals detected during the present study remains unclear, especially since brain AChE levels did not appear to be inhibited. Both techniques are generally regarded as complementry to each other in toxicological studies. For example, if brain AChE levels are depressed, the presence of insecticidal residues in body tissues is often used to confirm that brain AChE levels were influenced by the presence of an insecticide and not to some stress related physiological response. Laboratory studies have demonstrated that brain AChE levels are inhibited following exposure to insectides and have been confirmed with tissue residue analsyses. For the organophosphates diazinon and parathion, maximal brain AChE inhibition occurred six to 24 hrs following exposure (Montz and Kirkpatrick 1985). In other studies (Zinkl et al. 1980, Rattner and Hoffman 1984), brain AChE levels of mice have been inhibited by as much as 38% of the concurrent control values by Orthene and acephate. In the present study, the absence of any carbofuran-related AChE depression suggests that methods used to collect tissue samples should be closely monitored in a field situation rather than indicating that there was no direct effect on brain AChE levels.

It has become readily apparent that a significant information gap exists for studies relating to field applications of carbofuran. Results of the present study have confirmed the presence of carbofuran in the food chain (i.e. insecticide residues) and suggest that a carbofuran-induced population response occurred. The actual mechanism of intoxication is not clear but it is likely that small mammals in the Youngstown study area were ingesting carbofuran-contaminated foods. However, exposure through inhalation or

dermal contact routes is also possible (Stickel 1973, McEwen and Stephenson 1979). Based on the feeding habits of small mammals (Banfield 1974), vegetation and insects were likely major sources of carbofuran residues. Morris (1970) found that arthropods, when abundant, will form a considerable proportion of the diets of **P. maniculatus** in Saskatchewan.

Even though carbofuran is considered to be non-phytotoxic at insecticidal dosages, it has been known to stimulate some plant growth (McEwen and Stephenson 1979). Moreover, carbofuran is relatively persistent in soils, with it's half-life varying with pH (Getzin 1973). For example, the half-life of carbofuran in sandy soils is 30 days and up to 80 days in muck (McEwen and Stephenson 1979). In fact, low carbofuran residues can remain in soils for up to one year. Therefore, the potential exists for some bioaccumulation in plants and possibly indirectly affecting the relative availabilities of food and cover long after small mammals have been exposed to carbofuran. Rattner and Hoffman (1984) found that following Orthene treatments, brain AChE levels gradually recovered to pre-treatment levels coincident with the disappearance of Orthene residues on vegetation. Keith et al. (1959), Barrett and Darnell (1967) and others (Robel et al. 1972, Spencer and Barrett 1980) have all documented changes in small mammal species composition that have directly or indirectly resulted from insecticidal alterations in food and cover availabilities. Thus, even though carbamate and organophosphate insecticides are considered to be less persistent than the organochlorines, there is enough evidence to suggest that these insecticides can accumulate in a food chain (Hall and Kolbe 1980) and result in secondary poisoning of other vertebrates (Mendelssohn and Paz 1977, Hill and Mendenhall 1980, Balcomb 1983).

6.0 CONCLUSIONS AND RECOMMENDATIONS

Numerous studies involving the acute and subacute effects of carbamate, organophosphate and organochlorine insecticides on laboratory and field populations of small mammals have been undertaken. These studies indicate that small mammal respones to insecticides are influenced by a number of factors including species, type of insecticide, habitat type, degree of exposure and various physiological parameters. But research related to the potential effects of carbofuran on wildlife is lacking. This is particularly important in view of the highly variable population responses of small mammals to various groups of insecticides documented in other studies and because of carbofuran's high acute toxicity to wildlife. Small mammals appear to be particulary sensitive to carbofuran with an LD_{50} of only 2.0 mg of insecticide per kg of body weight.

Based on a review of relevant literature, only Zectran (a carbamate) and endrin (an organochlorine) has been shown to be more toxic than carbofuran. However, carbofuran is the only insecticide that is currently receiving widespread use in Alberta. Moreover, of the field studies that documented significant small mammal responses to insecticide use, all had higher LD_{50} values than carbofuran. In fact, two of the most common metabolites of the parent compound carbofuran (Metcalf et al. 1968) have lower LD_{50} values than many of the carbamate and organophosphate insecticides for which significant effects were reported. This implies that carbofuran has the potential to exert a strong toxic effect, even at prescribed field application rates. Toxicologists generally contend that dosage levels are more important than acute toxicity levels (McEwen 1982) suggesting that a highly toxic chemical applied at a low dosage is generally no more toxic than a chemical of low toxicity applied at a high rate. However, McEwen (1982) states that the validity of this concept depends on extreme precision in the formulation and application of highly toxic chemicals, particularly in a field situation. In Alberta, a detailed publication on uses, field application rates and handling and safety procedures of various insecticides is available (Alberta Agriculture 1985b), but the extent to which these guidelines are followed by landowners during field applications is not known. Therefore, it is possible that insecticides may be applied in the field at rates higher than the levels

recommended by manufacturers.

The present study was initially designed to provide a preliminary assessment of the possible effects of carbofuran use on naturally occurring, unconfined small mammal populations in southern Alberta and to evaluate field sampling and laboratory methodologies. Results of the 1986 field season indicate that the recommended field application rates of carbofuran resulted in some acute and subacute effects on small mammal populations in the Youngstown study area, however, the extent and specificity of carbofuran toxicity could not be determined. The study of toxicological effects of insecticides on naturally occuring small mammal populations requires detailed analyses of various population parameters such as density, reproduction, survival and recruitment rates. Therefore, more precise and accurate information under controlled field situations is now required to fully assess the acute, subacute and indirect long-term toxic effects of carbofuran exposure on the population dynamics and reproductive performance of small mammal communities.

Insecticides have the potential to exert significant effects on the demographic, behavioral and physiological characteristics of a small mammal population. These effects are magnified even further if secondary poisoning of other non-target species occurs. Because of the potential toxicological risks insecticide use may have on wildlife populations, a continued research effort is warranted. The research program should reflect the following considerations:

1. Design a more controlled field study potentially involving the use of small mammal enclosures similar to those described by Morris (1972).
2. Use of on-site laboratory equipment to facilitate tissue extraction for continued brain AChE determinations and residue analyses.
3. Determine the point at which small mammal population viability is affected.
4. Determine the effects of repeated carbofuran exposure on small mammal populations dynamics and reproduction.
5. Assess the implications of carbofuran exposure on multi-species small

mammal communities.

6. Evaluate the significance of carbofuran-contaminated small mammals as a potential exposure route (i.e. secondary poisoning) to raptors and carnivores.
7. Document habitat features and evaluate the importance of vegetation and arthropod residues as sources of carbofuran contamination to mice and other wildlife.

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